THE HEALTH STATUS OF TWO SPECIES OF TASMANIAN FARMED SHELLFISH, *CRASSOSTREA GIGAS* (THUNBERG, 1793) AND *OSTREA ANGASI* (SOWERBY, 1871).

by

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I, Joanne Ruth Wilson, declare that this thesis contains no material which has been accepted for the award of any other degree or diploma in any tertiary institution and that to the best of my knowledge and belief, the thesis contains no material previously published or written by another person, except when due reference is made in the text of the thesis.

ABSTRACT

A project to assess the health of Tasmania's farmed shellfish was conducted during the period October 1990 - June 1992. A total of 5290 Pacific oysters (*Crassostrea gigas*) and 630 flat oysters (*Ostrea angasi*) were collected during the program which involved near-monthly collections of shellfish from each of four growing areas in Tasmania. Pacific oysters were free of any prescribed or potential pathogen. Flat oysters were found to be infected with a serious pathogen, *Bonamia* sp, and a viral inclusion of unknown significance. Histological examination of these samples revealed the presence of low numbers of commensal organisms in the tissues of both species of oyster. Pacific oysters were infected with a viral infection of the gametes, rickettsial inclusions, two species of ciliates, two protozoans of unknown taxonomy, a turbellarian and two types of copepods. Flat oysters were infected with rickettsial inclusions, a ciliate and two types of copepod. Three species of spionid polychaetes were dissected from shellblisters affecting Pacific oysters. Changes in histological appearance of Pacific oysters including changes in the leydig tissue, the types and degree of infiltration of haemocytes and atrophy of digestive tubules show some seasonal trends and are correlated to the gonadal stage of the oyster. Also, digestive tubule atrophy and abundance of brown cells are correlated with lower salinity.

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CHAPTER 1: INTRODUCTION

Tasmania has a relatively small, but successful, oyster culture industry based primarily on the Pacific oyster (*Crassostrea gigas*) and, to a lesser extent, on the native flat oyster (*Ostrea angasi*). During 1991-1992, 2 850 035 dozen Pacific oysters (worth $9 975 122) and 18 972 dozen flat oysters (worth $85 374) were produced. The continued success of this industry will be greatly dependent on the health of its oyster stocks.

Disease has caused large-scale mortalities in cultured and wild stocks of oysters world-wide. This has led to at best, greatly reduced harvest or at worst, the complete collapse of some shellfish industries. Such disease events were first recorded in the 1920's (Farley, 1988) and continue to the present day. Much research effort has been directed into the etiology, life cycles of the causative agents, and techniques for diagnosis of such diseases. Indeed, more is known about diseases of commercial bivalve mollusc species than all other marine invertebrates combined (Lauckner, 1983).

The impact of disease is greatly increased when the disease is spread via translocation of infected oyster stocks to previously uninfected areas. Previously unexposed oysters are often more susceptible to introduced pathogens and the ability to transport live oysters between countries has meant that diseases can be spread to areas which would never have been exposed by natural dispersion of the etiological agents. Farley (pers. comm.) proposed that most of the mass mortalities that have occurred world-wide in oyster stocks are directly attributable to this action.

The movement of live animals between countries, states and even estuaries has always posed risks for disease introduction. Relatively unrestricted and/or clandestine movement of oyster stocks has occurred in the past with devastating results. Also disease has been spread after health testing failed to detect serious pathogens - an example being *Bonamia ostreae* (van Banning, 1985). Although these latter cases are rare, the consequences can be disastrous. Most countries now require strict, long term monitoring of the health of imported shellfish in order to minimise this risk. These regulations will increasingly apply to the importation of live shellfish for human consumption as well as for aquaculture purposes.

In addition to serious pathogens, oysters play host to a range of commensal organisms. Organisms considered commensals under some conditions may have a deleterious effect in high numbers, when the host is compromised or when previously unexposed shellfish are infected.

One of the difficulties in studying a disease outbreak is distinguishing between the normal types, prevalences, and distribution of presumptive commensal organisms and those organisms which cause disease. Also, normal seasonal variation in histological appearance of the tissue should be differentiated from pathology associated with disease. Once baseline information is known, abnormalities are more easily detected and etiological agents can be diagnosed more accurately. The study to be reported was undertaken to provide such data for Tasmanian oysters.

As little information was available relating to the health status of Tasmanian farmed shellfish, a health survey was conducted over the period October 1990 - April 1992 inclusive in which 5290 Pacific oysters and 630 native flat oysters, cultivated on leases throughout Tasmania, were examined macroscopically and histologically. In this, commensal organisms were identified and recorded, and organisms of special interest or of significance were further studied using electron microscopy. Also, variations in the histology of the tissues of Pacific oysters were recorded and related to environmental conditions and the reproductive stage of the population.

The aims of this project were:-

1. To assess the health status of stocks of Tasmanian cultured oysters and the significance of associated commensals and parasites.

2. To describe these commensals and parasites and their geographic and temporal distribution.

3. To assess and describe changes in non-reproductive tissues over the period of study and determine if these changes were related to environmental changes or the reproductive state of the oyster.

In this chapter, the literature pertaining to relevant oyster diseases, parasites and commensals and changes in the histological appearance of oysters is reviewed in

sections A and B respectively. In addition, surveys of shellfish health of a similar nature to this study are reviewed in section C.

A) Pathogens and commensals of marine bivalves.

Pathogens and commensal organisms of marine bivalves are represented by a diverse range of taxa from viruses to metazoa.

This chapter includes a review of the literature pertaining to oyster commensals, parasites and diseases of oysters. The content is restricted to organisms or diseases which were either found in farmed oysters in Tasmania or elsewhere in Australia or affect *C. gigas* or *Ostrea angasi* grown in other parts of the world. Hence, some significant oyster diseases e.g. MSX (*Haplosporidium nelsoni*) of *Crassostrea virginica* in USA and Aber disease (*Martelia refringens*) of *O. edulis* in Europe are not included here.

Viruses

Viruses are well known as disease causing agents in higher animals but have only recently been described from marine bivalves. Farley et al. (1972) were the first to record a viral infection of oysters. They reported a herpes-like virus from a small population of *Crassostrea virginica* in the USA. Intranuclear inclusions were observed within cells surrounding haemolymph sinuses. The affected population of oysters were exposed to elevated water temperatures and experienced 50% mortality.

Viruses were later reported from both "healthy" and diseased stocks of shellfish and the following discusses these reports.

Iridoviruses have been associated with mass mortalities of both adult (gill necrosis virus and haemocytic infection virus) and larval (oyster velar viral disease) Pacific oysters *(Crassostrea gigas)*.

It was originally reported that gill necrosis virus (GNV) and haemocytic infection virus (HIV) were diseases of *C. angulata* and were occasionally detected in *C. gigas*. However, *C. angulata* is now considered to be synonymous with *C. gigas* (Hine et al., 1992). Hence further discussions of GNV and HIV in this paper will refer to *C. gigas* as the host species.

GNV was first associated with large scale mortalities of oysters in France in 1967. Gross signs of the disease began with yellow-greenish pustules which developed into perforations and, eventually, ulceration of the gills. The virus described by Comps (1988) was an icosahedral particle 380 nm in diameter which developed in the cytoplasm of haemocytes or gill cells. Histologically, affected gill tissue showed tissue necrosis, infiltration of haemocytes, and changes to the structure of the gill filament. The disease was characterised by hypertrophy of the infected cell nuclei and occurrence of large polymorphic cells (Comps, 1988).

HIV was implicated in mass mortalities of *C. gigas* in French waters during the period 1970 - 1973. The disease was very similar to GNV in its morphology, and location in the cytoplasm of haemocytes or gill cells, but differed in its pathological effect (Comps, 1988). Signs of this disease included weakness of the adductor muscle and histologically, the presence of abnormal haemocytes, haemocytic infiltration and an increase in the number of brown cells in the leydig tissue.

Leibovitz et al. (1978) first reported a specific viral disease affecting hatchery-reared larvae of Pacific oysters. Known as oyster velar viral disease (OVVD) its diagnosis and management was described by Elston and Wilkinson (1985) after an 8 year study of a Washington hatchery. The disease derived its name from the effect it had on the velum which exhibited lesions and became distorted and deformed (Elston and Wilkinson, 1985). Losses were seasonal between March and June and caused up to 50% mortality in the hatchery studied. Examination of affected larvae using transmission electron microscopy (TEM) revealed intracytoplasmic inclusion bodies in velar, oral and esophageal epithelium. These contained virions of 228nm diameter which morphologically resembled an iridovirus. Although the importance of this finding was recognised it has not been experimentally established that oyster velar virus is the cause of hatchery losses.

Recently, reported mortalities of Pacific oyster larvae in hatcheries have been attributed to herpes virus infections. Mortalities of Pacific oyster larvae occurred during summer of 1991 in New Zealand (Hine et al., 1992) and France (Nicolas et al., 1992). In both cases the herpes-like viral particles were found in the tissue using electron microscopy. Nicolas et al. (1992) give little detail on mortality rates other than to say mortality and morbidity rates were abnormal. In the New Zealand case, the disease affected larvae 3-4 days after spawning when they

stopped feeding. Mortality was 60-100% after 7-11 days. Viral particles were visualised in hypertrophied fibroblastic cells in both cases and from phagocyte precursors (Hine et al., 1992) and cells adjacent to infected fibroblasts (Nicolas et al., 1992). Viral particles in these cells were reported by the French as 70±2 nm in diameter within the nucleus and 90±5nm in the cytoplasm (Nicolas et al., 1992) and New Zealand workers reported particles of 97±4nm within the nucleus of infected cells.

Meyers (1981) detected Cowdry Type A inclusions in the mantle of adult American oysters during routine surveying. Although electron microscopy studies to confirm the presence of viral particles in these inclusions were not undertaken, Cowdry Type A inclusions have previously been found to contain herpes-like virus particles.

Herpes-virus associated mortalities of shellfish often occur in conditions of elevated water temperature (Farley et al., 1972; Hine et al., 1992; Nicolas et al., 1992). Consequently, these viral infections may only be detrimental to their hosts under certain environmental conditions.

As well as being related to disease, viruses have also been reported from apparently healthy shellfish. Meyers (1979) isolated a reo-like virus from juvenile American oysters (*C. virginica*) on fish cell lines. The oysters were collected as part of a routine monitoring program and were not noted to be experiencing elevated mortality.

A virus morphologically resembling papovavirus has been reported from the germinal tissues of American oysters (*C. virginica*) (Farley, 1976). Infected cells were hypertrophied with large, basophilic, finely-granular nuclei and a small amount of cytoplasm. These abnormal nuclei contained virus particles of 53 nm diameter, without envelopes which suggested they were a type of papilloma virus probably a papovavirus. Papilloma viruses have oncogenic potential, however neoplasia was not associated with this infection. Rather, the infection was described as a lytic virus infection (Farley, 1976). Farley (1985) noted that female gametes are primarily affected with some infections in males. Infections were usually seen in small numbers of cells during the maturing stages of gametogenesis (Farley, 1985). This condition has been observed in a number of species (*Crassostrea virginica, C. gigas, C. rhizophorae, Saccostrea*

commercialis, and *Ostrea lurida*) and from a wide geographic range (Farley, 1985). Infected shellfish are apparently healthy.

Intranuclear inclusion bodies were discovered in the digestive gland of pearl oysters *Pinctada maxima* in northwestern Australia (Pass et al., 1988) during examination of oysters for the cause of mortalities. Viral particles found inside the inclusions were made up of subunits surrounded by an electron-lucent halo, and were unlike viral particles previously reported in molluscs. The inclusions were common in the population studied, and there was no evidence that the infections were related to disease or mortality.

Bacteria

There have been only a few reports suggesting that diseases of adult oysters can be attributed to the action of pathogenic bacteria. Pathological conditions caused by bacteria in Pacific oysters have primarily been reported from larval and juvenile stages in artificial intensive culture (Loosanoff and Davis, 1963; Brown, 1983; Garland et al., 1983). Bacterial agents isolated and identified as probable causative agents of disease were of the genera *Vibrio, Pseudomonas* and *Alteromonas* (Elston, 1984). Studies on such disease events are numerous and the reader is referred to Loosanoff and Davis (1963) and Brown (1983) for reviews of this literature.

Results of laboratory experiments by Grischkowsky and Liston, (1974) and Lipovsky and Chew, (1972) on adult American oysters (*C. virginica*) suggested that bacterial invasion leading to death was increased under conditions of elevated water temperatures.

This relationship between water temperature and bacterial infection also has implications for natural populations of oyster larvae as spawning is triggered by a rise in water temperature.

A serious mortality of adult pearl oysters in Western Australia was attributed to bacterial infection by *Vibrio harveyi*, normally a harmless component of the gut flora (Pass et al., 1987). Disease and mortality occurred after transport of adult stock from the collecting grounds to nearshore leases during which time they were exposed to high concentrations of bacteria and, during winter months, greater

differences in ambient water temperatures between the collecting grounds and the leases. Highest mortality occurred during winter although water temperatures were not considered cold (19 C). It was thought at this temperature, conditions were favourable for bacterial growth and the defence system of the oyster was depressed. Mortalities were reduced through changes in transportation and culture techniques.

Juvenile Pacific oysters on the Pacific coast of the USA are affected by a disease which is characterised by bacterial erosion of the hinge ligament (Dungan and Elston, 1988). Mortalities of up to 90% in one week have been associated with this disease. The bacteria isolated from affected hinge ligaments were described as Gram-negative, long, flexible rods, lacking flagella. Dungan et al. (1989) identified this bacteria as *Cytophaga* spp based on in-vitro studies. This study also demonstrated that the *Cytophaga*-like bacteria degraded oyster hinge ligament tissue in-vitro providing evidence that this bacteria is responsible for the disease.

Mantle lesions, often present in oysters with hinge ligament damage were thought to result directly from *Cytophaga* sp which caused the hinge ligament damage. However, tests have shown that the bacteria found in such lesions are morphologically distinct from *Cytophaga* spp (Dungan and Elston, 1988).

A second bacterial disease affects adult Pacific oysters on the Pacific coast of the USA, Canada and Japan. Pacific oyster nocardiosis has been called "fatal inflammatory bacteremia" and "focal necrosis" in USA and multiple abscesses in Japan (Elston et al., 1987). In most cases the mantle displayed raised yellow-greenish nodules. However Elston et al. (1987) suggested diagnosis is best made by histolgical examination as mantle abscesses are not always apparent. Gram-positive, acid-fast bacteria, of the genus *Nocardia* have been isolated from infected oysters and the disease reproduced in vitro in apparently healthy oysters injected with these isolates (Friedman and Hedrick, 1991). Adult *C. gigas* especially those grown in warm shallow embayments are affected, usually during the summer months. It appears to be a recurring problem in Matsushima Bay, Japan and in Puget Sound, USA. High water temperatures and elevated nutrient levels have been implicated as major factors this disease (Friedman and Hedrick, 1991).

Rickettsial Infections

Rickettsia, chlamydiae and mycoplasms have only recently been discovered in marine bivalves. Harshbarger et al., (1977) first described these prokaryotic organisms from clams (*Mercenaria mercenaria* and *Mya arenaria*) and oysters (*Crassostrea virginica*) from Chesapeake Bay. Since this initial report a wide range of marine bivalves from around the world have been reported to contain these inclusions. Inclusions often occur in digestive or branchial epithelia (Elston, 1986) and are not usually associated with disease or mortality in infected animals. They are found in the digestive epithelia of clams *Tellina tenuis* in Scotland, from razor clams *Siliqua patula* in USA, and oysters *Crassostrea virginica* in USA (Buchanan, 1978; Elston and Peacock, 1984; and Meyers, 1981) and in gill epithelia of clams *Tapes japonica* from USA and a scallop *Patinopectin yessoensis* and from soft shelled clams *Mya arenaria* from USA (Elston, 1986; Fries et al., 1991).

Reports of ricketsiales-like organisms *in C. gigas* have come from France (Comps et al., 1977), Canada (Blackbourne et al., 1990) USA (F. Kern, pers comm.) and Spain (Azevedo and Villalba, 1991). There have been no reported findings of rickettsiales-like organisms in *O. angasi.*

With the exception of work by Goggin and Lester (1990) on rickettsiales inclusions in giant clams on the Great Barrier Reef, research into or reporting of rickettsial inclusions in shellfish has been conducted in the northern hemisphere.

Whilst these infections often occur in healthy shellfish, even "harmless" infections may compromise the host and the effects are most evident when the host is stressed (Otto et al., 1979; Elston and Peacock, 1984; Goggin and Lester, 1990). In the most serious cases, the presence of rickettsiales-like organisms has been linked with high mortality and cellular damage.

Mass mortalities were linked to rickettsiales-like infections of branchial epithelium of scallops *Placopecten magellanicus* in USA (Gulka et al., 1983) and *Pecten maximus* in France (le Gall et al., 1988; 1991). Although the relationship between the rickettsiales-like organism and the mortality event has not been clearly established, there was evidence that such heavy infections caused physical damage and physiological stress on the host. Infected *Placopecten magellanicus*

exhibited degeneration of the adductor muscle and hypertrophy of infected gill cells (Gulka et al., 1983). le Gall et al. (1988) did not observe changes to the adductor muscle, but noted destruction of infected cells. An epizooitological study of the latter infection (le Gall et al., 1991) showed that an increase in intensity of infection in winter was closely followed by an increase in mortality.

At present, it is difficult to define the taxonomic status of the inclusions in molluscan bivalves. Techniques commonly applied in avian and mammalian infections such as serological tests and cell culture are not available for study of marine bivalve infections due to the absence of techniques specifically designed for the isolation of rickettsias from invertebrates and the lack of suitable cell lines on which to culture the organisms. Consequently, rickettsial organisms in bivalves are characterised by morpholgical features observed with light and transmission electron microscopy.

Fungi

A fungus, *Ostracoblabe implexa,* has been detected in a number of oyster species including *Crassostrea gigas*, *C. virginica* (Elston, 1990) and recently *C. cucullata* in India (Raghukumar and Lande, 1988) where little damage is caused to the host. However it has had a devastating effect on *Ostrea edulis* populations in Europe causing shell deformities which lead to the death of the oyster (Elston, 1990).

Initially the fungus obtains its nutrition from breakdown of the shell matrix and causes its host no damage. Once it burrows into the shell cavity the mantle secretes a concholin layer to "wall-off" the invading organism. This layer is in turn invaded by the fungus. Adductor muscles become affected as a protruding knob-like structure is formed by this process. As a result the adductor muscle is weakened and the oyster eventually dies (Alderman and Jones, 1971). The disease is greatly enhanced by water temperatures above 20°C.

The shell of bivalves, commonly used to collect spat, has been cited as the greatest source of infection of the fungus. The disease has been controlled by clearing dead shell before laying spat and chemical treatment of spat showing early signs of the disease (Elston, 1990).

Protozoa

Members of this Kingdom cause parasitic diseases of marine bivalves which are of considerable economic importance. There are 2 reasons for this:-

1. infections occur in commercially important stocks of bivalves.

2. many of these species are highly pathogenic to the host causing mass mortalities in affected populations.

Accordingly, there is much literature on these parasitic diseases. Despite the amount of study many aspects of these diseases such as their life cycles, are unknown.

Phylum Sarcomastigophora

The flagellate *Hexamita* was thought to act as a primary pathogen, but it is currently accepted that the organism invades opportunistically when the host is stressed by unfavourable environmental conditions or compromised by another disease (Shuster and Hillman, 1963 op. cit. Lauckner, 1983).

Phylum Apicomplexa

Perkinsus spp, protozoan parasites of shellfish, have often been responsible for losses in cultured shellfish populations. Although it has not been associated with disease in oyster populations in Australia, or described from *C. gigas* or *O. angasi* in other parts of the world, some discussion of this disease is warranted due to the devastating effect of *Perkinsus marinus* on oyster populations in USA and its widespread distribution and lack of host specificity in Australian molluscan species.

Perkinsus marinus (*syn. Labyrinthyomyxa marina, syn. Dermocystidium marinum*) has been described from populations of the American oyster *Crassostrea virginica* along the eastern seaboard of the USA where it is associated with mass mortality of commercial oyster stocks. This disease has been the subject of intensive study since its detection in 1949 (Mackin, Owen and Collier, 1950 op. cit. Perkins, 1969). The taxonomic position of this parasite has not yet been clearly established although Perkins has successfully argued for

its position in the Apicomplexa rather than the Sporozoa (see Canning, 1986). It has been found that the disease is greatly influenced by environmental factors and that prevalence and intensity of infection are increased under conditions of high temperature and salinity.

Perkinsus spp parasites have been detected in shellfish in Spain, Portugal, the Mediterranean and Hawaii (op. cit. Lester et al., 1990) and in a number of Australian molluscan species.

In Australia, *P. olseni* was first described from a stock of abalone (*Haliotis ruber*) experiencing mortality (Lester and Davis, 1981). Soon after, it was detected in a second species of abalone *H. laevigata* (Lester et al., 1990). In both cases, high temperatures were linked with increased numbers of parasites and a peak in mortality.

Perkinsus-like organisms have been described from scallops (*Pecten alba*) in Port Philip Bay, Victoria during examination of shellfish severely affected by a bloom of toxic algae (Parry et al., 1989). It was suggested that the infection may have caused the high mortalities in scallops observed at the time. However, determination of the effect of the *Perkinsus*-like organisms was confounded by the effect of the toxic algae. *Perkinsus* sp was also detected in *O. angasi* from Port Philip Bay on a separate occasion (Munday, pers. comm.). This infection occurred during a period of temperature stress with concurrent *Bonamia* sp infection.

Perkinsus spp were also found in bivalves from the Great Barrier Reef (Perkins 1985; op. cit. Lester et al., 1990). Further sampling of a number of bivalve species from numerous locations on the Great Barrier Reef showed this parasite was widespread. Moribund giant clams *Tridacna gigas* were infected with a *Perkinsus* spp although the cause of death could not be definitively determined. A number of other species which tested positive for the parasite were apparently healthy although these species may be acting as reservoirs (Goggin and Lester, 1987).

It is not known whether there is more than one *Perkinsus* species involved in these infections. Histologically differences in the morphology of the parasites in different hosts were observed. However, cross infection experiments with

Australian molluscs have shown a lack of host specificity for *Perkinsus* spp (Goggin et al., 1989).

In contrast, *Perkinsus marinus* was thought to be host specific due to failure of cross infection by inoculation (Lauckner, 1983). However, the target species were not exposed to the infective stage of the organism - the zoospores - during these experiments (Goggin et al., 1989).

Phylum Ascetospora

Included in this group are "famous" diseases as MSX (*Haplosporidium nelsoni*), Aber disease (*Martelia refringens*), haemocytic parasitosis *(Bonamia ostreae* and *Bonamia* sp.) and other "microcell" diseases eg Denman Island disease (recently classified as *Mikrocytos mackini*). Three, and possibly four, have been described in Australia - *Martelia sydneyi* (or QX Disease) and *Mikrocytos roughleyi* (winter mortality) from Sydney rock oysters, *Saccostrea commercialis* (Farley, Wolf and Elston, 1988), *Mikrocytos* sp in *Pinctada maxima* (Hine, pers. comm.), and *Bonamia* sp from native flat oysters (*Ostrea angasi*) (Rawlins, pers. comm; own observations). A detailed review of the diseases MSX and Aber disease are not included here (see introduction). However, the reader is referred to papers by Ford and Haskin (1982) for a comprehensive study of MSX in *C. virginica* from Delaware Bay, USA, and Figueras and Montes (1988) for a review of Aber disease in *O. edulis* in Europe.

Martelia sydneyi

"QX" disease has caused large scale mortalities (up to 95%) in Sydney rock oyster (*Saccostrea commercialis*) stocks in the subtropical area of its range. The disease is caused by a haplosporidian *Martelia sydneyi* which resembles *M. refringens*, a parasite of European flat oysters (*Ostrea edulis*) in France.

QX disease was first described from diseased oysters in southern Queensland (Wolf, 1972). Ultrastructural studies clarified its taxonomic position and it was named *Martelia sydneyi* (Perkins and Wolf, 1976). Oysters suffering from the disease were often very watery with most of the gonad resorbed, and had an enlarged pale digestive gland (Wolf, 1979). The parasites infected the digestive gland of oysters and could be diagnosed either by examination of histological

sections or from fresh smears of digestive gland tissue for the presence of the characteristic sporulating stages.

Proliferation of the parasite has been associated with dislodgement, disorganisation and sloughing of cells from the basal membrane of digestive epithelium. Wolf (1979) suggested that the oysters were probably unable to digest food material and starved to death.

The disease has only been reported in oysters from southern Queensland and northern New South Wales. Studies have shown that low temperatures retard the progression of the disease and that, although salinity seemed to have little effect once the disease was established, the timing of first infection was often at the time of the "first fresh" or low salinity event (Lester, 1986).

"Microcell" diseases

"Microcell" diseases include those caused by members of the genera *Mikrocytos* and *Bonamia*. Although the members of these two genera are similar - both are small protozoa between 1-4 μm in diameter which cause severe pathology and death in oysters-there are some important differences between the two genera. *Mikrocytos* sp parasites are associated with pustules or abscesses on the gonad and mantle, are found in the cells of the vesicular connective tissue and are only associated with localized haemocytic reaction (abscesses), and are found only in Crassostreid or cupped oysters. In contrast, *Bonamia* spp parasites typically cause wateryness and pale digestive gland of ostreid oysters, are located in the cytoplasmic vacuole (phagosome?) of haemocytes, and are usually associated with a diffuse, systemic haemocytic reaction. Some ultrastructural differences are also apparent. *Mikrocytos mackini* have spherical, eccentric nucleoli. In contrast *Bonamia* spp have a crescent shaped, peripheral nucleolus.

Mikrocytos roughleyi

Sydney rock oysters are also infected by a "microcell" *Mikrocytos roughleyi*, the cause of "winter mortality". Winter mortality is characterized by pustules, ulcerations and abscesses on the mantle, gonad and gill of the oyster and the impairment of adductor muscle function (Farley et al., 1988) An intense phagocytic infiltration of the connective tissue is associated with the parasite - a 2 μm organism with a nucleus of 1μm.

The disease has affected oysters in southern NSW and caused mortalities during winter months, in conditions of high salinity and low temperatures. Oysters less than 3 years have not been affected by the disease (Farley et al., 1988).

Mikrocytos mackini

Although not described from Australia, "Denman Island disease" is the only "microcell" diseases that affects *C. gigas*. Quayle (1961, op. cit. Bower, 1988) first observed the disease in 1960 on the east coast of Vancouver Island. Thirty four percent of oysters had died. Visual examination of the oysters revealed green pustules on the mantle surface and/or pus-filled sinuses. It was not until 1969 that histological examination revealed an etiological agent. Described as a "microcell" it was identified by Mackin as an intracellular organism, 1-3 µm long in connective tissue adjacent to abscesses (Farley et al., 1988). This "microcell" parasite has been recently classified as *Mikrocytos mackini* (Farley et al., 1988).

The disease appeared in mid-spring and ended in late summer. Highest mortalities were seen in oysters over 2 years of age and in those grown at low tide levels (Bower, 1988). In the initial outbreak, 25% of survivors had scars on the shell and body surface corresponding to pustules. Additionally, the gonads had a grey mottled appearance indicating abnormal gonad development and the digestive gland was beige instead of green in moribund oysters.

Monitoring of Denman Island Disease had continued since 1960 although early studies of prevalence have been based on the appearance of gross signs not accompanied by histological examination. Bower (1988) has shown that microcells are not always present in tissues where lesions are evident. Identical gross signs are evident in Pacific oysters with nocardiosis infections (Bower, 1988).

Bonamia spp

History and Pathology

It is probable that the first report of *Bonamia ostreae* was by Katkansky et al. (1969) who described a "microcell" in haemocytes of *Ostrea edulis* grown in California and associated the organism with mortalities and poor growth seen in

these stocks. These stocks had originated from Milford laboratory, Connecticut and were placed in four bays in California. Three of these replantings experienced high mortalities - up to 100% within 12 months in one stock and 91% after 3 years in another. All stocks experienced depressed growth. Histological examination revealed an organism 2-3 µm in diameter with a nucleus of 0.8 µm occurring within leucocytes or free in the blood. The similarity between this parasite and an organism infecting *Crassostrea gigas* in Canada was noted. Generalized infiltration of haemocytes especially around digestive tubules and reduction in height of digestive tubule epithelium was associated with the infection in *O. edulis*. Macroscopic inspection of infected oysters showed that they were thin, watery and transparent.

The disease bonamiosis or haemocytic parasitosis was first described by Pichot et al. (1979) after high mortalities were experienced in stocks of flat oysters in France. The parasite resembled closely the microcell described by Katkansky et al. (1969). French scientists described the cause of haemocytic parasitosis as a small spherical cell 2-3 µm diameter with a nucleus of 1 µm located in a cytoplasmic vacuole within the haemocytes (Grizel et al., 1988).

A second species of *Bonamia* (*Bonamia* sp) was discovered in stocks of Bluff oysters, *Tiostrea chilensis* (=*Tiostrea lutaria*) in New Zealand in 1986 (Dinamani et al., 1987). Histological examination of oysters from a population experiencing large scale mortalities revealed the presence of a *Bonamia*-like parasite. Based on electron microscopy and serological testing (Miahle et al., 1988) this parasite was considered sufficiently different to be classed as a separate species.

Recently a parasite morphologically similar to *Bonamia* sp was detected in *O. angasi* stocks from Victoria (Rawlins, pers comm) and in Tasmania (Handlinger, pers comm). Preliminary results of DNA probe tests suggest that the *Bonamia* sp from Victoria and New Zealand are identical, but different from *Bonamia ostreae* from France (Lester, pers comm.)

Surveys and experimental work has shown that mortalities occur attributed to *B. ostreae* or *Bonamia* sp throughout the year but are increased over the summer period (Balouet et al., 1983, Hine 1991a, Rawlins, pers. comm.).

Geographic Distribution (*Bonamia ostreae*)

France

Bonamia ostreae was first detected in France after abnormally high mortalities were reported from Tudy Island in July, 1979. By November of the same year it had also been found at other sites in the important oyster growing regions of Brittany (Balouet et al., 1983). Severe mortalities were reported in 1980 and 1981 and surveying of oyster populations in other areas revealed a widespread distribution of the parasite (Balouet et al., 1983).

Since the report of this disease by French workers, the discovery of this parasite in *O. edulis* stocks in Europe and USA has been rapid. Examination of mortalities of oysters or as part of routine screening has shown the presence of *B. ostreae* in the Netherlands, Denmark, England, Ireland, Spain and USA.

The spread of *B. ostreae* to previously unaffected areas is believed to be caused by translocation of infected shellfish. France imported oyster seed from a hatchery on the west coast of the USA now believed to be infected. This stock transmitted the disease to local beds and with movement of *O. edulis* to other parts of Europe, (intentionally/unintentionally) the geographic range of the parasite was dramatically increased, causing catastrophic losses in many areas.

Subsequent movements of stocks of infected oyster seed from France has spread this disease to other European countries.

England

Bonamia ostreae was first diagnosed in south west England in autumn 1982 (Bucke et al., 1984) after a serious mortality in *O. edulis*. Infected oysters had been relaid from the infected site to other oyster growing sites before *B. ostreae* had been diagnosed and thus the disease has been spread to other commercially important oyster beds (Bucke et al., 1984). It is thought that the disease was initially imported via infected seed from "the continent" (Hudson and Hill, 1991) but the scientific literature is not clear as to the country of origin.

Strict controls on the movements of oysters between oyster growing areas within England has minimized the spread of the disease although surveys have shown

that the disease is more widespread in cultured stocks than first thought (Hudson and Hill, 1991). This spread had probably occurred with translocation of infected stocks before the disease was diagnosed in 1982 and controls effected (Hudson and Hill, 1991). In some cases wild stocks became infected, but the natural spread of the disease seemed localized (Hudson and Hill, 1991). In two such wild stocks, the progression of the disease was slow, but after 3 years few live oysters could be found on these beds.

Ireland

A similar scenario occurred in Ireland where *B. ostreae* was first discovered in 1987. High mortalities in commercial oyster operations prompted investigation and histological examination revealed the presence of the parasite (Mc Ardle et al., 1991). Significant mortalities had been occurring since 1984 but these were attributed to predation (Mc Ardle et al., 1991). Analysis of frozen material pre-dating the initial diagnosis in 1987 revealed the presence of the *Bonamia ostreae* parasites in samples collected in summer 1986 (Rogan et al., 1991).

Three bays in Ireland have been found to be infected. Two of the sites were fished commercially and experienced high mortality, whilst the third natural bed, showed no evidence of large scale mortality (Mc Ardle et al., 1991). Thus far, studies have shown that natural beds of oysters in the vicinity of commercially exploited infected oyster beds, are free of the parasite.

Netherlands

The Netherlands had been importing flat oyster seed from France since 1963. Despite screening of imported oysters for disease since 1974, *B. ostreae* was introduced and was first detected 1980 in oysters from Brittany, France (van Banning, 1985). Oysters established in the commercial oyster growing area of Yerseke Bank showed a peak of infection 3-4 months later (van Banning, 1985).

Drastic measures were taken to eradicate the disease and to prevent its spread to other *Bonamia*-free areas. The importation of seed was stopped, as was transport of oysters. In addition, a program of dredging all flat oysters from Yerseke Bank was initiated (van Banning, 1985). A survey using *Bonamia*-free oysters as indicators, showed that after 3 years of dredging and eradication, the levels of infection were down to negligible levels (van Banning, 1986). In 1988, commercial farming of oysters transported from a *Bonamia*-free area, the

Grevelingen, was permitted. Unfortunately, infection levels of 8-71% at different sites of Yerseke Bank were detected and commercial operations were abandoned (van Banning, 1991).

The Grevelingen had, until 1988, been free of *B. ostreae* - as determined by regular testing. The introduction of *Bonamia ostreae* to this area has been a major blow to flat oyster culture in this country as it was reliant on the healthy Grevelingen stocks for its commercial operation. It is thought that *B. ostreae* was introduced to the area by infectious material probably via ships that had been working in the Yerseke Bank (van Banning, 1991). The Grevelingen is separated by a physical barrier from the Yerseke Bank and thus the only way that *Bonamia* could have entered is by transport of infectious material. Mortality of up to 80% (detectable prevalence of *Bonamia ostreae* 48%) has been observed at some sites.

A different disease management strategy has been employed in this area than on the Yerseke Bank which included research to determine the epizooitology of the disease. Initial results suggested that stress due to fishing and environmental factors has a large influence on the susceptibility of oysters to the disease. Also, areas remote from the central infection zone were free of the disease. Oddly, a population of oysters planted at a higher density showed a lower prevalence of *Bonamia* when compared with a lower density planting (van Banning, 1991). The statistical significance of these findings were not indicated, and thus it is possible that the observed differences in prevalence may have been due to local environmental conditions.

USA

Translocation of infected stocks has also been the cause of the spread of *Bonamia ostreae* to previously uninfected areas in the USA. Elston et al. (1986) suggested that *B. ostreae* had been introduced to Washington state via oyster seed imported from Elkhorn Slough in California.

A study of nine separate stocks of *Ostrea edulis* in western North America was undertaken by Elston et al. (1986). From these studies, it was found that in 4 of the Washington stocks, *B. ostreae* was present. Under experimental conditions, 3 of these stocks exhibited mortality of 30 - 35 %. One population that did not show any signs of mortality or illness was subsequently found to be infected

with *B. ostreae*. On-farm mortality figures were not given in this paper. Inflammatory reaction usually associated with *B. ostreae* was noted in 5 stocks, but no parasites were observed. *B. ostreae* was not detected in oysters from Humbolt Bay, California.

Friedman et al. (1989) documented the results of a survey conducted in Tomales Bay and Santa Barbara channel in California. *B. ostreae* was detected in low levels (3 -20%) in *O. edulis* in 3 of the 8 sites examined - 2 in Tomales Bay and one in Santa Barbara channel. A higher proportion of affected oysters was reported by Katkansky et al. (1969) in Tomales Bay.

Other species of shellfish (including *Mytilus edulis*, *Ostrea lurida* and *Crassostrea gigas*) growing adjacent to *Bonamia* infected *Ostrea edulis* were negative when tested for *Bonamia ostreae* (Friedman et al., 1989).

Bonamia sp

New Zealand

Bonamia sp has caused devastating losses in stocks of *Tiostrea chilensis* in New Zealand. Since 1986, the main oyster fishery in the Fouveaux Strait has suffered up to 90% mortality due to bonamiosis (Dinamani et al., 1987). Here the oyster industry is based on a dredge fishery. Quotas have been drastically reduced from 4000 to 400 bags/ship/season (Hine, pers comm).

Electron and light microscope studies of *Bonamia* sp. have shown an annual pattern of infection in the New Zealand oyster population and from these studies a tentative life cycle has been suggested (Hine 1991a, 1991b).

Two forms of the parasite were recognized by light microscopy - dense and clear forms, and the pattern of infection in different tissue types was described. In addition, both prevalence and intensity of infection was noted. A seasonal pattern involving three main phases and five developmental stages were described from light and electron microscopy observations.

1. Incubation phase: Occurred during spring (September - November) and was characterised by very low prevalence and intensity of parasites. Parasites were basophilic staining and were mainly of the dense form. They occurred in the

tissue just below the basal membrane of the gut. Two developmental stages were recognised by electron microscopy in this phase. Developmental stage 1 containing few haplosporosomes and dense ribosomes was small and electron dense. Stage 2 parasites were somewhat similar although the shape of the nuclei and cell was irregular, and it was noted that Golgi structures had detached from the nucleus.

2. Proliferation phase: The timing of the proliferation phase was related to the reproductive cycle of the oyster. In December, haemocytes migrating to resorb unspent male gonad material were found to be infected. During January, the oysters developed into the female phase of their cycle. Similarly, developed ova which were not spawned were resorbed by the haemocytes during January - April and these haemocytes became infected. Both prevalence and intensity of infection increased dramatically in all tissues during this time and it is thought that absorbed ovarian tissue provides an energy source for the parasite during this phase of rapid proliferation. At this time dense and binucleate forms were common and were often extracellular due to the lysing of infected haemocytes (Hine, 1991b). Until April, the parasites were large dense forms and were eosinophilic staining. After this time, the proportion of clear forms increased. The large dense forms corresponded to developmental stage 3 seen in electron microscopy. Similarly to developmental stage 2, these were irregular in shape, although, in addition, they contained multivesicular bodies and large arrays of smooth endoplasmic reticulum. Developmental stage 4 parasites (clear forms) were less dense, had larger numbers of mitochondria and haplosporosomes.

3. Plasmodial phase: Both the intensity and prevalence of parasites decreased from May until July (winter). In August (late winter) a large number of oysters infected with low numbers of organisms caused a peak in the prevalence of the parasite. The proportion of clear forms increased over this time. Electron microscopy showed that these forms (developmental stage 5) had a dense plasma membrane, large vacuoles and were necrotic. It appears that the parasites were undergoing the early stages of sporogony, but spore stages have not yet been observed. Hine (1991b) noted the similarity of these stages to patterns of infection by other haplosporidians and suggested that the spore stage may have been or is being lost due to the success of infection by dense forms.

It is thought that the dense forms are the infectious stage, which are ingested during feeding and subsequently burrow through the gut epithelium and infect

haemocytes below the basal membrane. Electron microscope studies have shown that the parasite has well developed lobopodia at this stage and actively move through the gut wall (Hine, pers. comm.). The release of parasites at the infectious stage by the host occurs through the gonad, kidney, digestive diverticula and gill from January to August (Hine, 1991a), and through the decomposing tissue of dead animals.

There are similarities between this and the life cycle described for *B. ostreae* by van Banning (1990). An incubation phase occurs during winter and spring in both cases but the incubation phase of *B. ostreae* is equivalent to the proliferation phase in *Bonamia sp.*. van Banning (1990) proposes that a stage of *B. ostreae* occurs within ovarian tissue although *B. ostreae* has only been observed in the haemocytes of *Ostrea edulis*. *Bonamia sp.* has only been observed in the haemocytes of *T. chilensis* and is associated with both male and female gondal development. It is suggested by van Banning (1990) that the reason why *B. ostreae* is only found in ostreid oysters is due to the adaptation of *Bonamia* to the protandrous life cycle characteristic of these molluscs. However, the reasoning behind such a presumption is not clear.

Australia

Bonamia sp was first reported from cultured stocks of *O. angasi* in Port Philip Bay, Victoria in 1991 (Rawlins, pers. comm.). Surveys have subsequently shown that the parasite is present in wild and cultivated *O. angasi* from an additional two bays in Victoria. The presence of *Bonamia* sp was associated with severe mortality in oysters over 2 years which had previously been attibuted to spawning stress. The pattern observed in New Zealand stocks of *Tiostrea chilensis* although focal accumulations of haemocytes around the gut and digestive gland were observed in winter - the time of least mortality (Rawlins, pers. comm.).

Bonamia sp was detected in cultivated and wild stocks of *O. angasi* in Tasmania in February 1992. However, it is not clear that mortality is associated with *Bonamia* sp infection. Pathology associated with *Bonamia* sp is very different to that described in New Zealand or Victoria, consisting of primarily focal lesions of haemocytes containing few parasites which are confined to the epithelial border of the gut, digestive gland or gill (own observations, Handlinger, pers. comm.).

Preliminary findings from surveys of flat oysters in other states of Australia include a tentative identification of *Bonamia* sp from *O. angasi* in Western Australia.

Control

Countries in which *Bonamia ostreae* has been found have employed different strategies when dealing with this disease, some of these control measures have been mentioned in previous paragraphs.

Despite the identification of a parasite associated with mortalities (later identified as *B. ostreae*) in *O. edulis* stocks in USA, transfers of stock continued to France. After the devastating mortalities in oyster stocks there and the identification of the parasite in 1980, subsequent transfers of seed have spread the disease through areas of Europe and more recently the Mediterranean (Elston, 1990). In contrast to the accelerated spread induced by human movement of live oysters, natural spread of the disease was slow and very localized. Hence, the best way to prevent the introduction of the disease into "uninfected" areas is to prevent the translocation of infected stocks (Elston, 1990). Although *B. ostreae* is rarely detected in seed or juvenile oysters, transfers of such stock has resulted in a disease outbreak some time later. Thus infected broodstock should not be used as a source of seed for translocation. It follows then, that when testing stock for the presence of *B. ostreae*, older stock (e.g. broodstock) they should be tested at the time it is most likely to be detectable i.e. late summer or autumn.

Where *B. ostreae* is already established a number of measures can be taken to reduce the impact of the disease. Although some countries, notably England and The Netherlands, have tried to eradicate the disease by dredging flat oyster populations, this does not seem to be a successful strategy.

In England, an oyster clearance program began in 1986 to remove infected oysters from beds in the Beaulieu River. However, beds downstream from this operation were positive when tested in 1988. These beds were also cleared but in 1989 wild oysters 3 km downstream were also found to be infected. In both cases, downstream beds had previously been tested and had returned negative results (Hudson and Hill, 1991).

As mentioned earlier, the Netherlands embarked on a rigorous eradication program to rid the Yerseke Bank of bonamiosis. Three years of dredging followed by two years of experimental plantings yielded some promising results. However, when oysters were planted commercially, it was clear that bonamiosis was still present in the bay and commercial harvesting of oysters was stopped (van Banning, 1991). Stocks which succumbed to the highest mortality in the 1988 commercial planting had been bagged up and placed in storage basins for several weeks or months. This treatment had probably stressed the oysters (van Banning, 1991).

"Stress" appeared to have a marked effect on the incidence of the disease in a Dutch population (van Banning, 1990). Hudson and Hill (1991) stated that "flat oyster resistance to *Bonamia ostreae* appears to be significantly affected by dredging, handling and transfer of oysters and their exposure to air, low temperature and fluctuations in salinity".

Management strategies have been adopted in order to minimize the impact of this parasite. Some success has been achieved by on-growing oysters in the same region from which spat were collected and reducing the density of spat planted on beds (Grizel et al., 1988). These authors also mentioned the collection and translocation of spat from *B. ostreae*-infected areas to other oyster beds. This seems to be an unwise management strategy as it is through exactly this action that the parasite was introduced to France.

Although tests have not detected *B. ostreae* in other molluscan species from infected areas, some countries restrict the movement of all bivalve species due to the risk of parasites being carried in mantle fluid or newly settled flat oysters on the shells of the bivalve species to be translocated. However, the large infective dose required to establish infection in new hosts suggests that infection by mantle fluid is not a significant risk (Hine, pers. comm.).

Culture methods have been modified to reduce mortalities caused by bonamiosis. Oysters grown in off-bottom and subtidal culture have experienced fewer mortalities (Elston, 1990). Experimental work in England has shown that oysters held off the bottom on tressles or racks had a lower prevalence of the disease - 4% in tressles compared with 26% on the adjacent affected bed (Bucke et al., 1984). This conflicts with the findings of Katkansky et al. (1969) who found

high mortality among populations which had been held on wire racks above the bottom.

The transmission of the disease has been reduced by lowering stock density.

There have been conflicting reports as to the distance an infective parasite is able to travel. Tests in England showed uninfected oysters held 900m from an infected bed became infected at a prevalence of 4% (Bucke et al., 1984). However, it was not clear where these "*Bonamia*-free" oysters originated and so may have come from a stock subsequently found to be infected. It has been suggested by Hine (pers. comm.) that the parasites can only survive for a short time outside the host. It has been recommended that a "corridor" of 100m between infected and uninfected stock is enough to prevent infection.

An experiment was conducted in a *Bonamia*-infected area in France to determine whether interspersing *O. edulis* with *C. gigas* reduced the infection rate or mortality of flat oyster stocks due to *B. ostreae* infection. At low densities survival of flat oysters was improved by interspersing with *C. gigas*. However, the difference in mortality rates may be due to the reduced number of flat oysters per bag rather than the relative proportions of oyster species in each bag (le Bec et al., 1991). In addition, results may have been inaccurate as different methods for detection of *B. ostreae* were used at different times. One of the methods used (ELISA test) has subsequently been shown to be inaccurate (Hine, pers. comm.).

Impact on Other Species of Shellfish

In areas where *B. ostreae* has been detected, some examination of other species of molluscs in the area is usually undertaken. In all such cases, only other species of Ostreid oysters are found to be similarly infected. Mussels, clams and crassostreid oysters eg *C. gigas* have remained apparently free from disease or infection (Friedman et al., 1989). Bucke et al. (1984) noted mortalities in *Tiostrea chilensis* (= *Ostrea lutaria*) populations present in *B. ostreae*-infected regions in England. Histological examination revealed 16% prevalence of the parasite.

Taxonomy

The taxonomic position of *Bonamia* spp has not been clearly defined. Placement of *Bonamia ostreae* and *Bonamia sp.* into the Phylum Acetospora was based on the presence of haplosporosomes and multinucleate plasmodia seen in other genera belonging to this group (Perkins, 1988).

However Elston et al. (1987) argued that it was not appropriate to classify *Bonamia* spp in Phylum Acetospora as haplosporosomes and multinucleate plasmodia are also found in the myxozoa. In addition, as a spore stage of *Bonamia* spp has not been detected, *Bonamia* spp cannot be classified on the basis of spore structure. Indeed, Sprague (1979) in a review of the taxonomy of the haplosporidians in the Phylum Acetospora stressed how important spore structure is in defining the taxonomic position of such organisms. Perkins (1988) proposed that *Bonamia* spp were not members of the myxozoa based on the absence of the "cells within cells" structure characteristic of this group.

Diagnosis

Macroscopic signs of the disease have been described as erosion of the gill filaments with yellow banding of the gills (Grizel at al., 1988), a pale digestive gland, and thin, watery specimens (Katkansky et al., 1969). As these signs have been associated with other diseases or conditions in oysters, diagnosis of *B. ostreae* is achieved by histological examination or examination of blood or heart smears. Although extensive infiltration of haemocytes in connective tissue around digestive tubules has been reported as characteristic of this disease, this reaction may be due again to factors not associated with bonamiosis. Confirmatory diagnosis therefore can only be made on the presence of the parasites within the haemocytes or in the extracellular spaces of the oyster.

In some cases the cellular reaction resembling a response to *Bonamia* infection has been observed but parasites have not been detected. Hence, light infections have been very difficult to diagnose and the detectable prevalence is often less than the mortality rate observed.

New techniques of isolation and purification of the parasite (Miahle et al., 1988) led to the development of a serological test designed for rapid and accurate diagnosis of the disease (Grizel et al., 1988). However, the test has been shown

to be unreliable (Hine, pers. comm.) and the technique published initially for isolation and purification of parasites was incomplete and thus not able to be used by other researchers. Recently, the complete methods for this technique have become available through the publication of a Ph.D. thesis (Chagot, 1989). The isolation of pure suspensions of parasites has proved valuable in some aspects of research e.g. infectivity trials and will no doubt will be an important tool in further studies of bonamiosis.

Phylum Ciliata

Ciliates have been found in a wide range of bivalve molluscs. The majority of these are harmless commensals of the class Kinetofragminophorea e.g. *Ancistrocoma* and other thigmotrichid ciliates which have been commonly found in the mantle cavity, gill surfaces or, rarely, in the digestive tract of marine bivalves.
Although they have not been considered pathogenic, in large numbers they may compete for food, reduce water flow over the gills or cause irritation to which the host is likely to respond by producing mucus (Lauckner, 1983).

Pauley et al., (1967) have found gills and palps of *C. gigas* heavily infected with thigmotrichid ciliates in individuals with artificially weakened adductor muscles. Pathology associated with those infections included haemocytic infiltration, necrotic and liquified tissue. The authors suggested that these ciliates are secondary invaders as heavy infections were associated with weakened hosts. Healthy oysters exhibited low numbers of parasites which produced only minor tissue changes.

Descriptions of ancistrocomid ciliates commonly found in clams and mussels can be found in Kozloff (1946), Khan (1956) and Lom and Kozloff (1968). The latter two references provide ultrastructural detail of these organisms.

Rhynchodida, another group of trichodinid ciliates, use a suctorial tube to attach to the epithelial cells of the host and feed on their contents. Usually the epithelium of the gills and palps are affected, but the digestive system may also be targeted.

Lauckner (1983) considered the role of these organisms to be underestimated and that the presence of large numbers may have irritative effects similar to those

caused by peritrichous ciliates. This latter group included species such as *Trichodina*, *Uceolaria* and *Leithocha* which are commonly associated with bivalves. There has been some controversy regarding pathogenicity of *Trichodina*. Some authors have considered it a harmless commensal where as others have regarded them as parasitic. Large scale mortalities of recently settled *Cardium edule* have been associated with *Trichodina* infections (Lauckner, 1983) and experimental evidence cited in Lauckner (1983) supported the latter theory. It may be that a number of factors acting in concert may alter host-parasite equilibrium so that *Trichodina* becomes a harmful parasite.

Metazoa

Phylum Platyhelminthes

Predominantly, members of this phylum are free-living predators although a number of species from a range of orders have developed symbiotic, usually commensal, associations with invertebrates and some lower vertebrates (Jennings, 1971).

Parasitism and commensalism in the turbellaria has been reviewed by Jennings (1971) who noted that symbiotic turbellarians show a host type specificity i.e. members of one turbellarian family are associated exclusively with one type of host. Two genera (*Paravortex* and *Graffillia*) of the family Graffillidae (Suborder Lecithophora: Dalyelliodia) have been associated with bivalve and gastropod molluscs. Three species of *Paravortex* have been described from the gut and kidneys of bivalve molluscs. Morphological characteristics thought to be adaptive for parasitism (loss of ciliation, surface epithelial pigment and eye spots) have also been noted in free living species. However, physiological changes in feeding, digestion and blood biochemistry described by Jennings and Philips (1978) are adaptive to this symbiotic mode of life.

Paravortex scrobiculariae have been described from *Scrobicularia plana* and *P. cardii* from *Cardium edule* in Britain (Freeman, 1957) and *P. gemellipara* from three species of shellfish *Geukensia demissa*, and *Ischadium recurvum* and *Mytilopsis leucopheata* on the eastern seaboard of the USA (Wardle, 1980). Prevalence of these infections in host populations can be high (70.4% in *Scrobicularia plana* infected by *P. scrobiculariae* and 88.2% of *Cerastoderma edule* infected by *P. cardii*) (Jennings and Philips, 1978). However these infections occurred in apparently healthy hosts. Similarly, Wardle (1980) reported that infected hosts appeared healthy.

Some genera are more harmful as predators of oysters, especially spat. High mortalities of cultured *O. edulis, C. virginica* and *C. gigas* in the USA are attributed to predation by one species *Pseudostylochus ostrophagus* (Wolke, 1957; op. cit. Lauckner, 1983).

Phylum Helminthes

Trematodes

Lauckner (1983) stated that digenetic trematodes are the most important metazoan parasites of bivalve molluscs. Bivalves have primarily acted as intermediate and/or transport hosts and rarely as final hosts. Larval stages of trematodes of the family Buchephalidiae have been found commonly in a number of oyster species. *Bucephalus cuculus* have been decribed in *C. virginica* (Burton, 1961; Gauthier et al., 1990) at prevalences of 7.2% and 0.5%. The parasite invaded the gonad tissue causing castration and sporocysts invaded much of the other tissue. The host tissue was severely depleted and the infected animals probably died (Lauckner, 1983).

Adult stages of the genus *Proctoces* have been found in oysters including *C. virginica* (Couch, 1985).

Cestodes

Larval stages of the marine cestode *Tylocephalum* have been found in *C. virginica* (Couch, 1985). *C. virginica* displayed a severe host response and walling off of the parasite, although the oyster appeared healthy (Cake and Menzel, 1980; op. cit. Lauckner, 1983).

Nematodes

The larval stages of nematodes have occasionally been reported from oysters where their presence caused little damage to the host. They have been found in healthy *C. gigas* (Dinamani, 1986) and *C. virginica* (Burton, 1961; Couch, 1985; Gauthier et al., 1990). *Echinocepholus sinensis* has been found in the reproductive tracts of female *C. gigas* where some tissue response was noted (Cheng, 1975; op. cit. Lauckner, 1983).

Phylum Polychaeta

Some members of the Class Polychaeta (Family Spionidae) have been described as oyster "pests". Two genera in particular, *Polydora* and *Boccardia*, have been found to cause shell blisters in a number of species of bivalve molluscs.

Infestation by these worms has in some cases been associated with mass mortalities of shellfish stocks, although some authors have reported little or no damage to affected populations or individuals.

Perhaps the first reports of the damaging effect of *Polydora* species on oysters were given by Whitlegge (1890) and Roughley (1925). Mass mortalities of the Sydney rock oyster *Saccostrea commercialis* were attributed to infestation by *Polydora ciliata*. (Re-examination of the data has since confirmed *P. websteri* and not *P. ciliata* to be the invading organism (Blake and Evans, 1973)).

As a result of these studies and recommendations, oysters grown in New South Wales estuaries are cultured in racks and on sticks suspended off the bottom to reduce this mudworm infestation.

Methods to reduce mudworm have had variable success. Loosanoff and Engle (1943) experimented with American oysters in an area where shell blisters caused by *P. websteri* were known to occur. Oysters were suspended from the bottom in subtidal culture racks for 2 & 1/2 years at which time they were examined for evidence of shellblisters and mortalities recorded. Negligible mortalities were seen even though nearly all shells of 5 different age groups were infested with shell blisters caused by *P. websteri*. Comparable samples from the benthos were not as heavily infected. It is interesting to note that the oysters grown in suspended culture were unusually fat and growth rates were better than for the benthic sample.

Throughout the literature there is conflicting evidence on the damaging effects of spionid polychaetes. Lunz (1940) proposed that mudworm infection affects the oyster by restricting its living space and causing general weakening. Also, in economic terms, losses were incurred as the oysters were unsaleable due to their appearance. Decreased shell growth and appearance of stunted oysters has been attributed to *Polydora* infections (Cole and Waugh, 1956). Abscesses on the flesh of oysters have been caused by shell blisters (Lauckner, 1983). In contrast, a number of authors were unable to find any damage associated with mudworm infection (Loosanoff and Engle, 1943 and Stephen, 1978). Lauckner (1983) suggested that this variation may reflect differences in host - species interactions.

A number of spionid polychaete species have been reported from cultivated oysters in Australia and New Zealand. As mentioned above Whitlegge (1890)

and Roughleyi (1925) noted the presence of and damage caused by *Polydora websteri* in *Saccostrea commercialis* in New South Wales. Blake and Kudenov (1978) provide a comprehensive review and taxonomic study of the polychaete fauna including Family Spionidae, of south eastern Australia. Skeel (1979) examined the spionid polychaete fauna of a number of Australian molluscs including *S. commercialis*, *Mytilus edulis*, *Crassostrea gigas* and *Placopecten meridionalis*. *Polydora websteri* was associated with all these molluscan hosts and was the most damaging species especially for *S. commercialis*. Other species of spionid polychaetes found during this study were *P. haswelli*, *P. hoplura*, and *Boccardia chilensis*. Of these, only *P. haswelli* was found to cause shellblisters. *Boccardia chilensis* and *P. hoplura* were found on the external surface of the shell and were considered commensal species.

Pregenzer (1983) noted five species of spionid polychaete (*Polydora haswelli*, *P. hoplura*, *P. websteri*, *Boccardia chilensis* and *B. polybranchia*) associated with cultivated and wild mussels in southern Australia. Infection levels were generally low although more severe infections (when more than 10% of the population was severely affected) occurred in three sites. These severe infections occurred in silty areas or when mussels were taken from the benthos. A number of authors have suggested that shellfish grown in these conditions are more liable to infection by polydorids (op. cit. Pregenzer, 1983). Skeel (1979) has suggested that oysters grown in intertidal areas have lower polydorid infection rates due to the desiccation of the burrow during low tide. The results of recent studies in New Zealand on polydorid infections in *C. gigas* grown intertidally show that mud availability may be a factor in polydorid settlement (Handley, pers. comm).

A survey of parasites and symbionts of cultivated *Crassostrea gigas* in New Zealand by Dinamani (1986) revealed the presence of polydorid worms (probably *Polydora* sp) which were causing shellblisters. The shellblisters appeared to have little effect on the host population and there was little correlation between shell and meat damage and the degree of infection by polydorids.

The mode of entry of *Polydora* species into oyster shells has been the subject of many studies and much scientific debate. Discussions have included speculation on the role of the modified setae on the 5th setiger in formation of a burrow. It is now apparent that *P. websteri*, which burrows through the shell, does so by production of acid and subsequent dissolution of the shell. The modified setae

are not used for mechanical burrowing, but are used to maintain the inner detritus tube laid down by the worm (Heigler, 1969, op cit. Lauckner, 1983).

The reader is referred to Lauckner (1983) and Blake and Evans (1973) for a detailed account of the burrowing methods of and shell blisters caused by polydorids. However a brief description can be given here. The worm burrows through the shell at right angles to the surface forming a U-shaped tube. When this tube reaches the cavity between the shell and the mantle, the oyster puts down a layer of shell enclosing the worm and any associated mud. This is a reaction by the oyster to prevent damage caused by the accumulated mud or by mechanical action of the worm.

Variations include entry via the shell margin, again forming a U-shaped tube covered with shell by the oyster. In all cases, the worm has communication and access to the outside via 2 openings often with a "chimney" of mud protruding from each. It is thought that this extra production of concholin and nacre to form a blister places a metabolic stress on the oyster making the oyster more susceptible to other infections.

A large body of literature exists describing the biology and ecology of spionid polychaetes (Loosanoff and Engle, 1943; Hartman, 1941; Hopkins, 1958; Blake and Evans, 1973; Skeel, 1979). A number of authors have reviewed the taxonomy of spionid polychaetes. Most notably Hartman (1941) and Loosanoff and Engle (1943) have provided complete and accurate descriptions of most spionid polychaete fauna. Larval development and reproductive biology of *Polydora* species is given by Blake (1969).

The effect of freshwater on spawning and settlement of spionid polychaetes has not gone unnoticed. Loosanoff and Engle (1943) observed that a lowering of salinity may induce settlement of polydorid larvae while Stephen (1978) noted that spawning of *P. ciliata* occurs with a lowering of salinity in Indian waters. Stephen (1978) also noted that the conditions caused by monsoon rains which cause the salinity to fall to almost 0 % were detrimental to *P. ciliata* and can kill adults.

Reproductive strategies of the same species can change depending on the temperature of the environment. Tropical populations of, for example, *P. websteri* are broadcasters ie. they release many larvae at an early developmental

stage to the plankton. Conversely temperate populations hold the young in the egg cases where early hatchlings devour remaining eggs and are released at a later developmental stage. Hence the term brooders to describe this group (Skeel, 1979).

Phylum Crustacea

A range of parasitic copepods primarily in the order Cyclopoidia have been described in marine bivalves as commensal organisms on the gills or in the gastrointestinal tract of the host.

However, *Mytilicola intestinalis* is a notable exception to this rule. "Red worm" disease was thought to be the cause of large scale mortalities of mussel populations in England and Europe (Lauckner, 1983). It is uncertain however that *Mytilicola* was the responsible pathogen. Usually during periods of mortality, oysters were examined for the presence of the copepod to the exclusion of other potential disease investigation (Lauckner, 1983).

Another species, *Mytilicola orientalis* inhabits *C. gigas* and mussels in Japan and throughout the Pacific. It was spread with oyster seed to USA where it now inhabits a number of bivalve species. Unlike *M. intestinalis* infections, adverse effects on the host can be observed. Condition index was measurably lower although no reduction in growth or increase in mortality was seen in studies by Chew et al. (1965), Katkansky (1968) or Sparks et al. (1968) (op. cit. Lauckner, 1983). Also, metaplastic changes to the gut of *C. gigas* were observed especially from "gapers". Moore et al. (1978, op. cit. Lauckner, 1983) found that *M. orientalis* had completely eroded the gut mucosa and an appendage was seen invading the connective tissue. Fibrosis sometimes associated with these infections may be an attempt by the host to wall off the organism.

Pseudomyicola spinosus has been found in the mantle cavity and tissues of a large number of bivalve species. They have commonly been recorded in oyster populations from Australia and New Zealand and are generally regarded as commensals. The palps and gut of *C. glomerata* in New Zealand were infected with *Pseudomyicola spinosis* where the presence of the parasite was not associated with damage to the gills (Dinamani and Gordon, 1974, op cit. Lauckner, 1983). However, injury to the gut epithelium was observed in oysters where the copepod was present in the alimentary tract. This species was also

found in cultivated *Crassostrea gigas* in New Zealand by Dinamani (1986). These organisms were common in the mantle cavity and within the folds of the labial palps and were noted in histological sections invading the gut and digestive gland. Where the organisms were within the lumen of the gut, no host response was observed. A range of life history stages of the copepod were seen in oysters and has been suggested that the copepods may complete their life cycle within the mantle cavity of the oyster (Dinamani, 1986).

Pseudomyicola spinosus was found to be widely distributed in stocks of mussels (*Mytilus edulis*) in Australia (Pregenzer, 1983). In this study, *P. spinosus* was the only one of four copepods associated with mantle cavity or tissues of mussels to be seen inside the gut. Few specimens were seen in samples collected from Hobart compared with other sampling stations in Australia and it was supposed that temperature may be a factor limiting its geographic distribution. Another copepod *Lichomolgus uncus* was found commonly in the mantle cavity of mussels from Hobart region but few were found elsewhere. Another two as yet undescribed species of copepod (*Myicola* spp) were found to be associated with mussels although these were not very abundant. The latter three species were never seen in the gut of mussels.

Parry et al. (1989) noted copepods in the stomach and digestive diverticula of mussels in Port Philip Bay. Although these were not identified it was suggested that they were probably *Pseudomyicola spinosus*. A maximum of 10% of mussels were infected and there was never more than one copepod seen in each histological section.

P. spinosus was detected in the gut of *C. gigas* from Tasmania submitted for certification testing (Langdon and Humphrey, pers. comm.).

B. Changes in histological appearance

In addition to research on commensals and pathogens associated with a shellfish population, information on the normal seasonal histological variation in the tissues is valuable for health assessment. Often the pathologist is alerted to the presence of a disease or parasite by the associated pathology or host response. Also, changes in the histological appearance of the tissue may vary seasonally in relation to gametogenic cycles (Meyers, 1981) in response to environmental conditions (Gauthier et al., 1990) or in response to exposure to pollution or toxins (Couch, 1985).

Thus, it is important to be able to differentiate between "common normal structure and function and common malstructure and dysfunction" so that the causes of abnormal or diseased states can be more easily determined (Couch, 1988).

Despite the importance of this research for assessment of shellfish health the relationship between the histological appearance of oyster tissues and seasonal and environmental factors is poorly understood.

Typically, baseline studies of any type are poorly funded (Parry et al., 1989) and research on shellfish "health" has, in the past, concentrated on studies of disease events because of their economic and social consequences.

In addition, many long term studies on the effect of environmental conditions and seasonal effect on oyster tissues was related to growth rates and condition index determined by ratios of wet and dry weights of oyster tissue rather than histological examination.

Early studies of oyster pathology examined inflammation and wound repair in molluscs which had been injected with substances such as talc and turpentine (Pauley and Sparks, 1965; 1966 op. cit. Sparks, 1985), or had suffered a physical injury (Pauley and Sparks, 1967). These were performed with a view to understanding the processes of host response to a pathogen, and mechanisms of tissue repair. Sparks (1985) has reviewed the literature relating to inflammation and wound repair in molluscs.

More recently, prospective surveys of shellfish health have included studies of changes in the histological appearance of the tissue including connective tissue edema, focal or diffuse haemocytic infiltration, and digestive tubule atrophy. Studies in the USA by Meyers (1981), Couch (1985) and Gauthier et al. (1990) and in New Zealand by Dinamani (1986) have included information on histological changes, environmental conditions or parasite loads. The detailed results of these studies are discussed later in section C in conjunction with the parasitological findings of these studies. However, a brief general account of their finding can be given here.

Conditions regarded as indicators of poor general health included edema or disorganisation of connective tissue, increase in haemocytic infiltration either of a focal or diffuse nature, increase in numbers of brown or pigment cells and reduction in height of digestive tubule epithelium (digestive tubule atrophy). In many cases, these conditions occurred when oysters were spawning or soon after (Meyers, 1981; Dinamani, 1986). Gauthier et al. (1990) found that digestive tubule atrophy was correlated with salinity with atrophic changes being more prominent at lower salinities.

Blooms of toxic algae, especially dinoflagellates, have caused ill health and mortalities in shellfish populations in many parts of the world (Sparks, 1985).

Changes in the histological appearance of oyster tissue can be influenced by many factors. Therefore it is important to examine environmental conditions and physiological state of the oyster in order to determine the cause of such changes in a particular case.

The following section reviews the published surveys of shellfish health conducted in USA, Canada and New Zealand.

C. Surveys of Shellfish Health

Similar baseline studies of shellfish health have been conducted in the USA and Canada to assess the health of the shellfish population and the significance of the associated parasites.

Burton (1961) examined American oysters from the Chesapeake Bay region during spring and summer of 1959 and 1960. Two pathogens MSX (*Haplosporidium nelsoni*) and Dermo (*Perkinsus marinus*) were found in a sample taken in autumn 1960. A number of non-pathogenic and commensal parasites including *Ancistrocoma* sp, *Hexamita* sp, *Bucephalus cuculus*, and *Nematopsis* sp were also detected. The author noted that numbers of oyster parasites in the upper Chesapeake Bay were relatively low and reported a new site of infection of MSX. Additionally, focal accumulations of haemocytes were noted although no apparent cause was detected.

American oysters in the Chesapeake and Chincoteague Bays were later surveyed by Otto et al. (1979) who also examined hard clams and soft-shelled clams. Levels of MSX and Dermo were low relative to findings of earlier studies of these diseases. A number of parasites considered commensals were found in these shellfish including the ciliates, *Ancistrocoma* and *Sphenophyra* spp., the gregarine *Nematopsis ostrearum*, and rickettsiales inclusions (described as amorphous basophilic inclusions). Types and prevalences of rickettsiales organisms were similar in oysters from different environmental conditions and no significant geographic or seasonal trends were observed with regard to these infections.

A similar range of organisms was found in a two year survey of American oysters in Connecticut waters (Newman, 1971). Plasmodial stages of *Haplosporidium* (*Minchinia*) spp., possibly *H. nelsoni* (MSX) and *H. costalis* were found in low numbers of oysters and most infections were light. A neoplastic disorder of unknown significance was detected in one oyster. Commensal organisms included ciliates of the Family Ancistrocomidae on the gills and in the digestive gland, and *Sphenophyrea* sp, *Nematopsis ostrearum* and an amoeboid organism in the gut epithelium. A papovavirus infection of ova (ovacystis) were observed in some oysters. Previous studies had shown this condition was geographically widespread and affected only a small number of eggs in an oyster.

Meyers (1981) examined the parasites associated with cultured juvenile and adult *C. virginica* and with hard clams, *Mercenaria mercenaria* in New York. A range of organisms were found in histological studies of the shellfish. Of these, an actinomycete and some plasmodial organisms, (possibly *Haplosporidium* spp.) were of potential significance to shellfish populations. Both of these organisms were only found at one sampling site. The haplosporidian caused appreciable host pathology although no host response was associated with the actinomycete infection. Because of the effect of these organisms on oyster populations elsewhere, they were considered potentially important pathogens. Commensal organisms similar to those reported in previous surveys were found in *C. virginica* including the ciliates *Sphenophyra* sp., and *Ancistrocoma* sp., rickettsiales inclusions and ovacystis. In addition, intranuclear inclusion bodies, a sporozoan, another (unidentified) protozoan, a turbellarian, an arthropod and a proliferative disorder were recorded. All of these commensal or non-pathogenic organisms were found in low numbers and were not associated with mortality.

Clams were infected with a ciliate, a gregarine, rickettsiales inclusions and one had a mantle papilloma. None of these invoked any host response or were considered of pathological significance.

In addition to the parasitological findings, it was found that changes in the histological appearance of the tissues of American oysters were related to the reproductive cycle of the oyster. Oedema of connective tissue and infiltration of haemocytes into connective tissue occurred at the time of, or soon after, spawning. The pattern of change in digestive tubule epithelial height differed between sampling sites. In one site, the height of digestive tubule epithelia decreased during the time of spawning and recovered soon after. At the second site, the opposite was observed. However, in both cases, digestive tubule epithelial height was reduced during times of connective tissue oedema. The author suggested that these seasonal variations should be taken into account when assessing the histological profile of shellfish.

The effect of pollution on the diseases and parasites of *C. virginica* in 3 estuaries of the Gulf of Mexico was investigated by Couch (1985). Higher prevalence of disease was found in shellfish near heavily industrialised sites although a pollution related epizootic was not evident at the time of study. Disorders were classified as infectious or non infectious. Infectious disorders included the pathogen, *Perkinsus marinus* (present in a low number of oysters) and a number

of commensals including ciliates in the gills, rickettsiales infections, four species of helminth and nematodes. Non-infectious disorders related to histological profiles of oysters. It was supposed that digestive tubule atrophy was related to local environmental conditions as differences in pattern and prevalence of the condition between estuaries were evident. Seasonal trends in the pattern of haemocytic infiltration were not evident and probably related to the pathophysiological state (i.e. degree of stress) of the oyster.

Gauthier et al. (1990) examined levels of parasitism in *C. virginica* in 3 Louisiana estuaries and found that oysters were infected with the pathogen *Perkinsus marinus*, and a number of commensal organisms including *Nematopsis* spp, *Bucephalus cuculus*, rickettsiales-like infections, two Ancistrocomid ciliates, *Sphenophyra* sp and nematodes. Digestive tubule atrophy was also noted. Higher levels of parasitism and intensified digestive tubule atrophy were found in areas of higher salinity. Previous studies had shown that the prevalence and intensity of *Perkinsus* infections was greater in conditions of higher salinity. However, the influence of salinity on prevalence of the other organisms described in this study were not well studied. The authors also found a positive correlation between numbers of oysters infected with *Perkinsus marinus* and numbers with atrophied digestive tubules or parasitised by *Nematopsis ostrearum*. Oysters weakened by *Perkinsus* infection may have been more susceptible to other infections although the degree of digestive tubule atrophy may not have been due directly to *Perkinsus* infection. Other studies have shown that such changes in digestive tubules can be related to seasonal cycles (Meyers, 1981).

Blackbourne et al. (1990) summarised the findings of 5 years of studies on the parasites and symbionts of Pacific oysters (*Crassostrea gigas*) in British Columbia, Canada. Manila clams (*Tapes phillipinarium*) and scallops (*Patinopecten yessoensis*) were also examined as part of this survey. A wide range of commensal organisms detected included rickettsiales inclusions, *Ostracoblabe*-like fungus, ciliates, flagellates, gregarines, coccidia, turbellarians, trematodes, nematodes, polychaetes and crustaceans. A number of pathogens were identified from *C. gigas* including Denman Island disease (*Mikrocytos mackini*), an actinomycete infection (*Nocardia* sp) and hinge ligament disease (*Cytophaga* sp) and scallops were infected with two unidentified protozoan parasites. The geographic range of Denman Is. disease was limited to the waters around a small island (Denman Is.) and management strategies detailed in Bower

(1988) were developed to minimise the impact of this disease. Actinomycete infections occurred only in conditions of high water temperature.

Few baseline studies have been carried out in the southern hemisphere with the exception of Dinamani (1986) who conducted a survey of the parasites associated with Pacific oysters (*C. gigas*) in New Zealand and a study by Pregenzer (1983) which examined the metazoan symbionts of mussels (*Mytilus edulis*) in southern Australia.

Pacific oysters in New Zealand were found to harbour a range of protozoan and metazoan parasites in the mantle fluid and tissue. Disease was not associated with the presence of these parasites which included ciliates probably of the family Thigmophyra, nematodes, the copepod *Pseudomyicola spinosus* and spionid polychaetes identified as *Polydora* sp. In addition, changes in the histological profiles of oysters (including the degree of haemocytic infiltration, digestive tubule atrophy and disorganisation of connective tissue) were noted. In the absence of any pathogen or other causative agent, variations in these conditions were thought to be related to seasonal cycles.

The metazoan parasites of Australian mussels found in the mantle fluid and during microscopic dissection of tissues included pea crabs (*Pinnotheres hickmani*), copepods, trematodes, and spionid polychaetes (Pregenzer, 1983). The distribution of different species of these types of organisms at different sites around Australia were noted. Two species of copepod, *Pseudomyicola spinosus* and *Lichomolgus uncus*, 5 species of polydorid polychaetes and the trematode, *Gymnophallus* sp were associated with mussels in Tasmania (Pregenzer, 1983). It was suggested that the ranges of trematodes and some species of copepods within Australia were influenced by temperature and that polychaete infection could be reduced by growing mussels in clear water off the bottom. Pea crabs, *Pinnotheres hickmani* were more prevalent in mussels from Tasmania and Victoria than at other sites. Their presence in mussels has been linked with poor meat condition in earlier studies. With the exception of pea crabs, the presence of the other parasites was not associated with poor condition or disease of infested mussels.

CHAPTER 2. MATERIALS AND METHODS

2.1 Sampling Program

Four main growing areas encompassing all oyster leases in Tasmania were identified. The basis for the divisions were the geography of the coastline, and the similarity of ecological and physical parameters of each area (R. Brown, pers. comm.).

The four discrete growing areas are listed below:

Area 1 - Northwest and King Island
Area 2 - East coast
Area 3 - Southeast
Area 4 - D'Entrecasteaux Channel and Bruny Island

The location of these areas and oyster leases in Tasmania is shown in Fig 2.1.

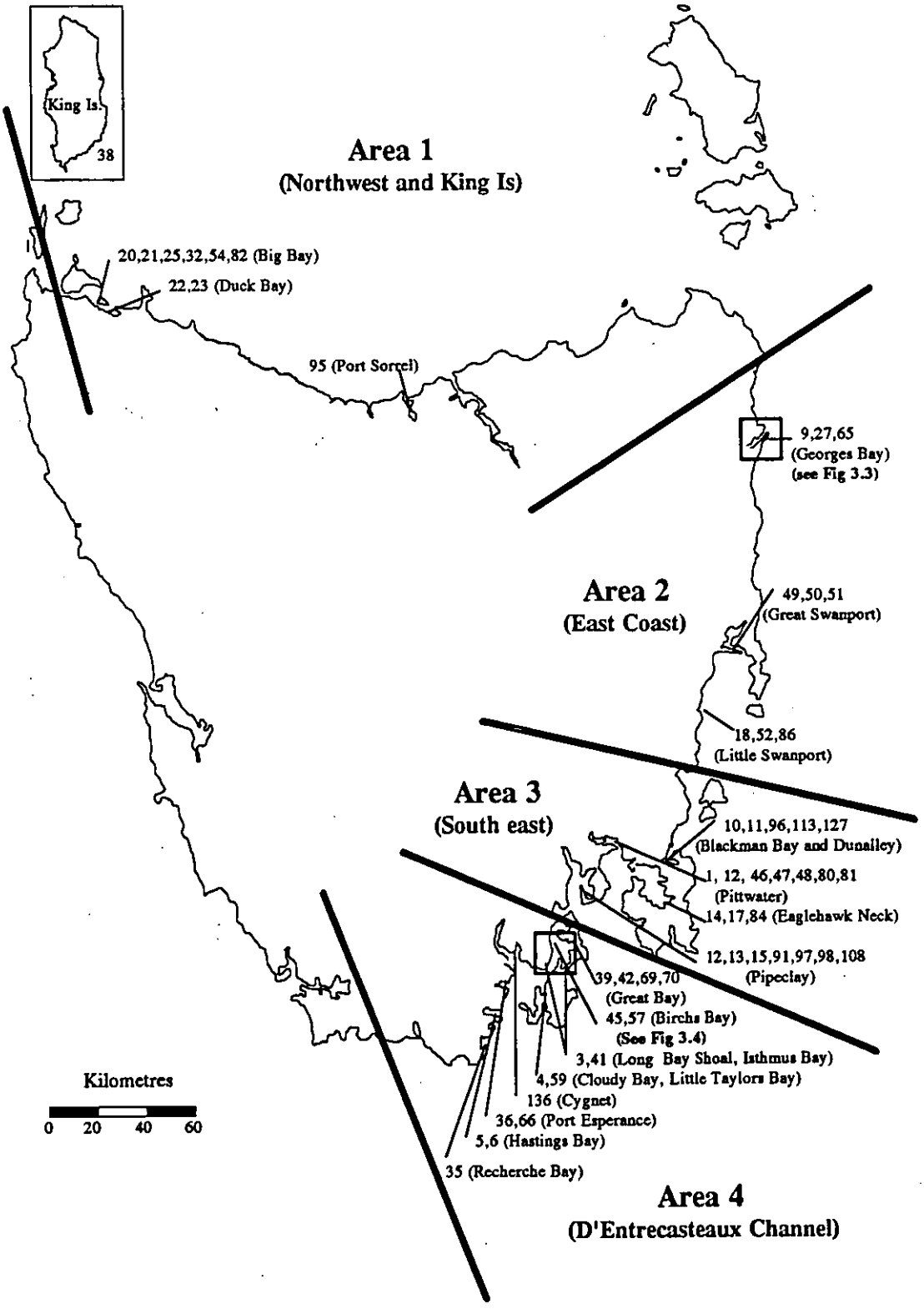
A list of areas, lease numbers and sites is included in Appendix 1.

Chronological listing of growing areas, leases and samples collected is presented in Appendix 2. Briefly, a sample of shellfish was collected from each of the four growing areas on a near monthly basis. Monthly samples were collected in October 1990, December 1990 - February 1991, April - July 1991, September - November 1991 and January - April 1992.

The monthly sample of shellfish was selected from a locality within the growing area. A locality was defined as a body of water in which farms were in immediate proximity to each other or were sharing a common body of water. If the locality contained one lease then all shellfish were taken from that lease. Where more than one lease occurred in a locality, the collection of the sample was divided between those leases. Localities were sampled on a rotational basis which ensured that individual leases were sampled at least twice during the survey and sometimes more often (see Appendix 2).

Two age groups for both species of oyster were identified. For Pacific oysters the age groups were 0-1 year ("juvenile") and 1-2 years ("mature") as most reach harvest size within 2 years. For flat oysters age classes were generally 1-

Fig 2.1: Location of oyster leases and growing areas in Tasmania



2 years ("juvenile") and 3-4 years ("mature") as harvest size is usually achieved within 3-4 years.

Samples contained equal numbers of both "juvenile" and "mature" age groups. Where one/two age groups were present 50/100 shellfish were taken respectively. Marine farms usually held more than one age group of oyster and so a sample from a growing area usually contained 100 shellfish.

A sample consisted primarily of Pacific oysters due to the predominance of this species in cultivation in Tasmania. Flat oysters were also collected where they were present on the lease or in the locality being sampled. The number of each species sampled reflected the proportion of that species on the farm/s being sampled. A minimum of 10 flat oysters of each age group was included in a sample of 100 shellfish. However, once *Bonamia* sp had been detected in Victoria, larger samples (40 flat oysters) were collected over the period November 1991 - February 1992 in addition to 100 Pacific oysters.

An extraordinary sample of 180 flat oysters was collected on 14.4.92 from two farms in close proximity to wild beds of oysters known to be infected with *Bonamia* sp. The contents of this sample is detailed in Table 2.1.

Table 2.1: Samples of *Bonamia* sp infected *O. angasi* collected from two leases in Georges Bay on 14.4.92.

Lease	Code	n	Age	Est. Mort	Source
A	1	30	>2yrs	20%	wild beds
	2	30	>2yrs	40%	wild beds
B	1	20	<2yrs	15%	wild beds
	2	40	>2yrs	15%	wild beds
	3	60	>2yrs	?	natural set

Ostrea angasi on lease A were harvested from wild beds of oysters within Georges Bay and were held intertidally. Oysters had spent up to 1 year on the lease.

Samples B_1 and B_2 had similarly been harvested from infected wild beds and had spent approximately 4 months on the lease. Sample B_3 consisted of oysters

oysters.

A total of 5290 Pacific oysters and 630 flat oysters were collected during the survey.

Live shellfish were sampled randomly from the leases usually at low tide and were collected either by myself, staff on the Shellfish Quality Assurance Program (Department of Health, Tasmania) or by selected farmers.

In most cases, samples were processed within 24 hours and in all cases within 72 hours of collection. Upon collection samples were immediately placed on ice and, on arrival at Mt Pleasant Laboratories, were refrigerated at 4°C. A code was assigned to each group for future referencing and filing.

2.2 Visual Examination

Any fouling or other marine fauna on the external surface of the shell was noted. In some cases, polychaete fauna which was present was fixed for later identification.

The oysters were opened at the hinge end with an oyster knife and the adductor muscle was cut away from both valves using a scalpel blade. Meats were examined for any sign of pustule, wateriness, emaciation, discolouration or deformity. Similarly, the shell was examined for signs of mantle recession, mud worm, pustules or other growth abnormalities. If any of these conditions were found they were described and, in most cases, photographed.

Blistered shells were separated and a number of characteristics describing the shellblister were recorded including thickness of the blister, the content of the blister, and its shape and size. Any polychaete worms present were dissected from these blisters using a scalpel blade to break away the blister and fine jewellers forceps (No 5) to tease the worm from the mud. Specimens were fixed in 7% formalin/seawater for 48 hours, rinsed twice in distilled water and finally stored in 70% alcohol. This prevented the spines of the polychaetes (which are important in taxonomic studies) becoming clogged with formalin precipitate while allowing adequate fixation not achievable by using 70% alcohol alone.
The polychaete worms collected between August 1990 and June 1991 were identified by Ms Lexie Walker (formerly of University of New England,

Northern Rivers, Lismore).

All shells, other than those with blisters, were discarded. If the flesh of the oyster appeared normal it was treated in the following way.

All animals were bissected at a routine position on the oyster as described by Howard and Smith (1983). This was the position approximately halfway between the adductor muscle, and the point at which the labial palps meet the gills (Fig 2.2).

This plane of section gave an optimum range of organs to examine histologically, namely the gut, including lobes of the intestine and digestive diverticula, gonad, gills and mantle. The anterior portion, that is from the point of bisection to the umbo end of the meat, was fixed in Davidson's fixative for 48 - 72 hours and then tissues were transferred to 70% alcohol.

From approximately a third of the shellfish in each sample, the posterior portion consisting of the gills, hind gut, and the adductor muscle was dissected for gut parasites. The remaining tissue was discarded.

Tissues were pooled in groups of 10 - 20 for fixation except where significant gross abnormalities were detected. In these cases, animals were fixed separately and assigned a separate code.

2.3 Histological Processing and Examination

After fixation, a section approximately 4-5mm thick was trimmed from the fixed tissue using a 150mm trimming knife. The oyster was oriented so that the umbo end was to the right with the gills pointing towards the operator. The first cut was made close to the cut face so that a smooth face was achieved. The second cut was made 4-5mm anterior to the first cut and this section was then placed face down in a plastic histological cassette. Tissues were stored in 70% alcohol until routine processing for paraffin embedding using a vacuum infiltration processor (Shandon Hypercenter XP) followed by sectioning at 4-5µmusingarotarymicrotome.

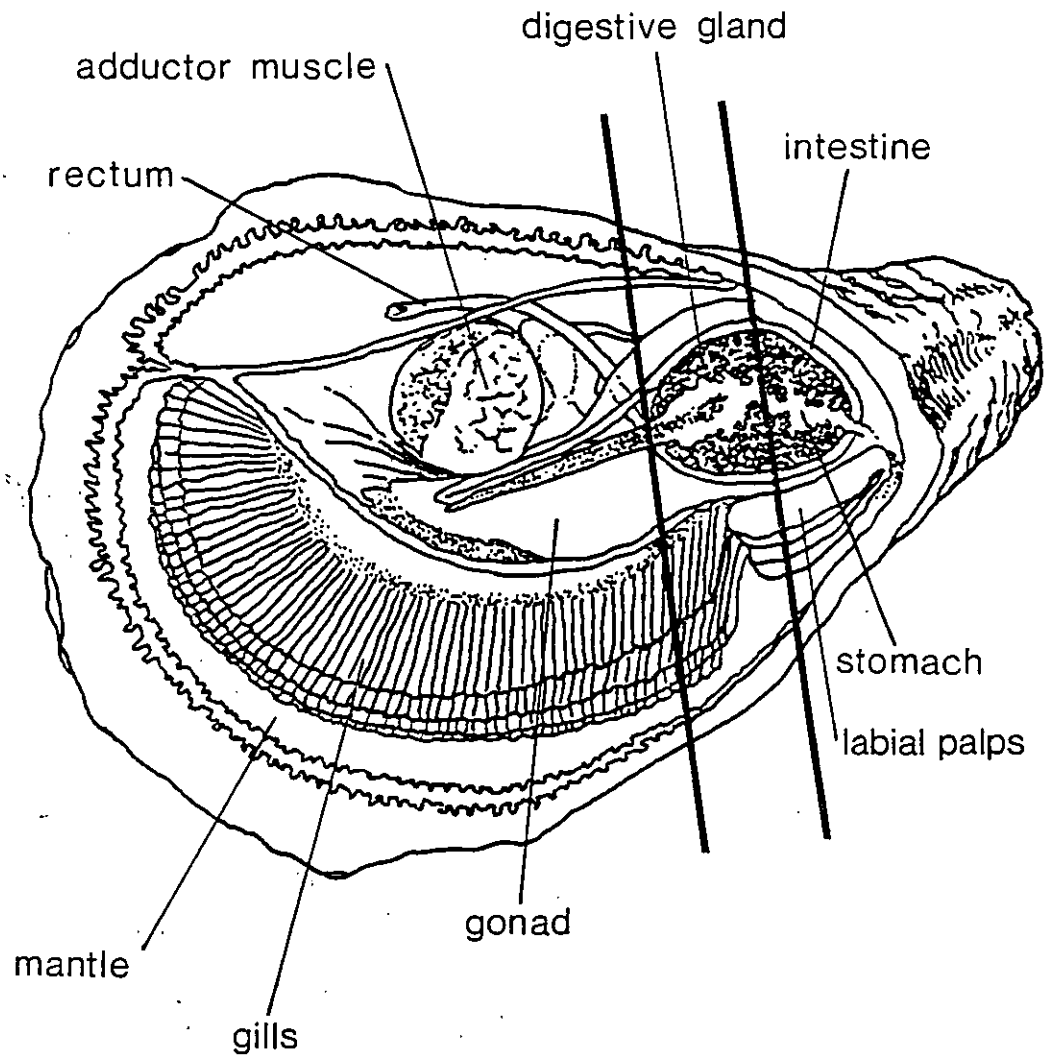


Fig 2.2: **Anatomy of an oyster. Bold parallel lines show the location of the cross section taken for histological examination (taken from Fig 28, Howard and Smith, 1983).**

Sections were stained routinely with Mayers haematoxylin and alcoholic eosin using an automatic stainer. Special stains including Gram, periodic acid Schiff (PAS), Giemsa and Gomori methenamine silver stains were also used in specific cases.

Histological slides were examined using a Nikon Labophot 2 microscope. Photomicrographs were taken using a Leitz Diaplan high power microscope mounted with a Wild Leitz camera mount and photoautomat.

Commensals and Parasites

Parasites observed during scanning of sections were assigned a type number for ease of recording. Numbers of parasites were expressed as *percent prevalence* where:

$$\% \text{ prevalence} = \frac{\text{n oysters containing the commensal}}{\text{n oysters in sample}} \times 100$$

Changes in histological appearance of Pacific oysters

A scoring system to describe tissue changes was established based on criteria used by other researchers to assess the health of an oyster (Meyers, 1981; Couch, 1985). Results for a particular score in a sample was expressed as a percentage of the total sample for changes in the leydig tissue, degree of haemocytic infiltration and proportion of brown cells present.

For example:

$$\text{Average prevalence (\%)} = 100 \times \frac{\text{n (leydig 0)}}{\text{n (leydig 0)} + \text{n (leydig 1)} + \text{n (leydig 2)}}$$

Leydig tissue

The degree of oedema or depletion of the leydig tissue (glycogen storage) cells in the section was scored LEY 0 (full) (Fig 2.3), LEY 1 (partially depleted) (Fig 2.4) and LEY 2 (majority of cells depleted) (Fig 2.5).

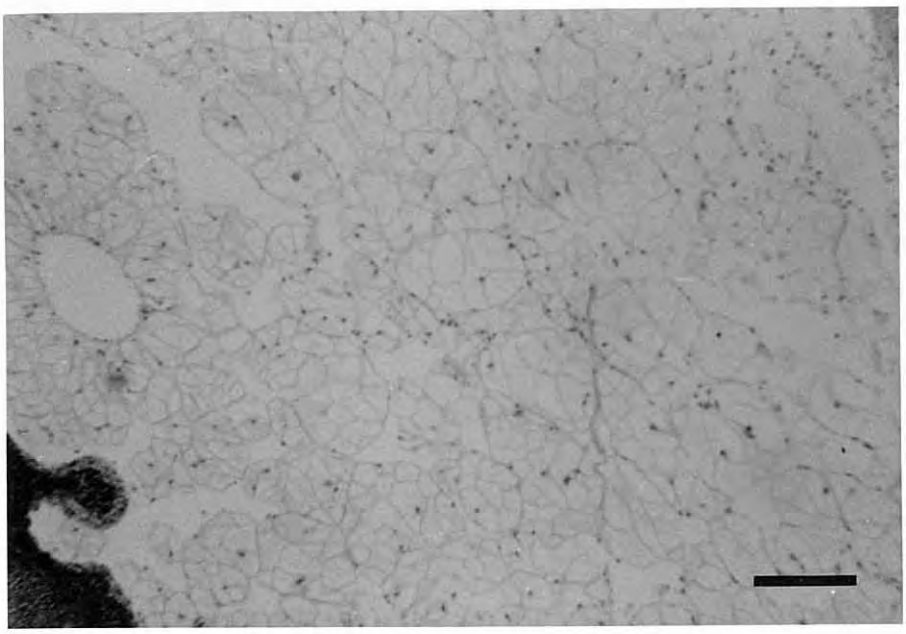


Fig 2.3: Section through connective tissue of *Crassotrea gigas* scored as "LEY 0". Note connective tissue cells appear "full" and oedema is not apparent (H&E). Scale bar = 100 μm.

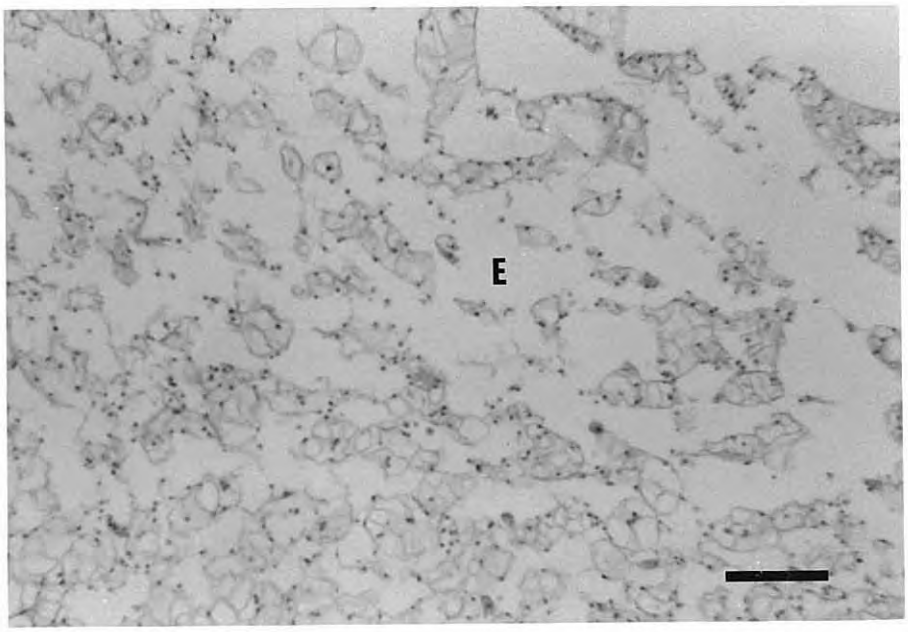


Fig 2.4: Section through connective tissue of *Crassostrea gigas* scored as "LEY 1" showing some evidence of oedema. Connective tissue cells are narrow and extracelluar spaces (E) are apparent between the cells (H&E). Scale bar = 100 μm.

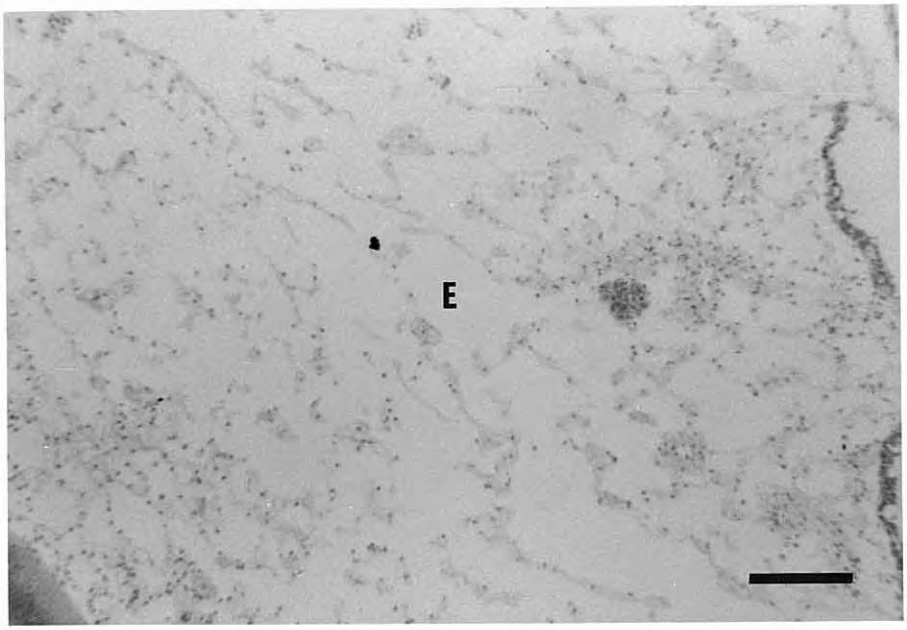


Fig 2.5: Connective tissue of *Crassostrea gigas* scored as "LEY 2" with severe oedema, large extracellular spaces (E) and disorganised appearance (H&E). Scale bar = 100 μm.

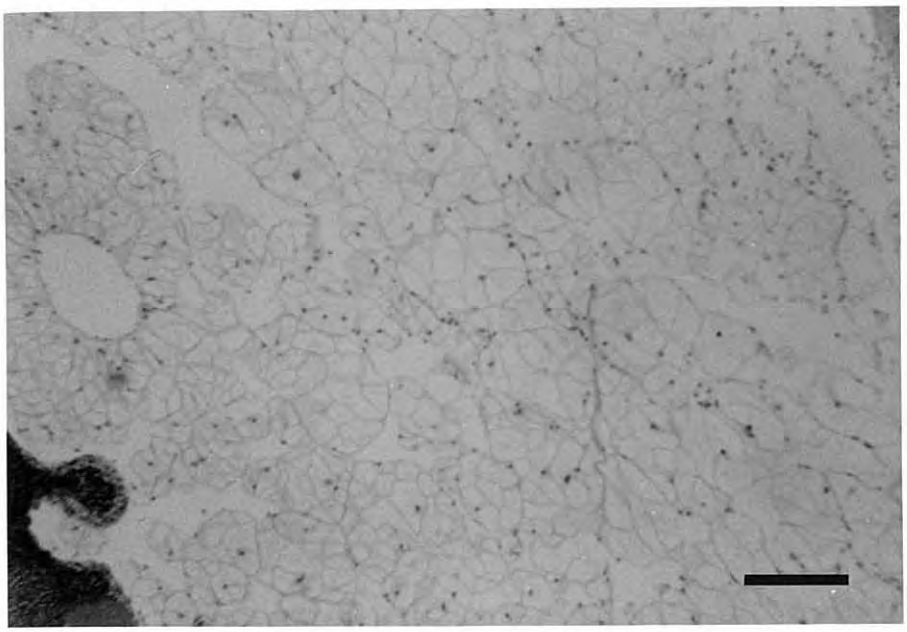


Fig 2.6: Section through connective tissue of *Crassostrea gigas* with minimal infiltration of haemocytes, scored as "HC 0" (H&E). Scale bar = 100 μm.

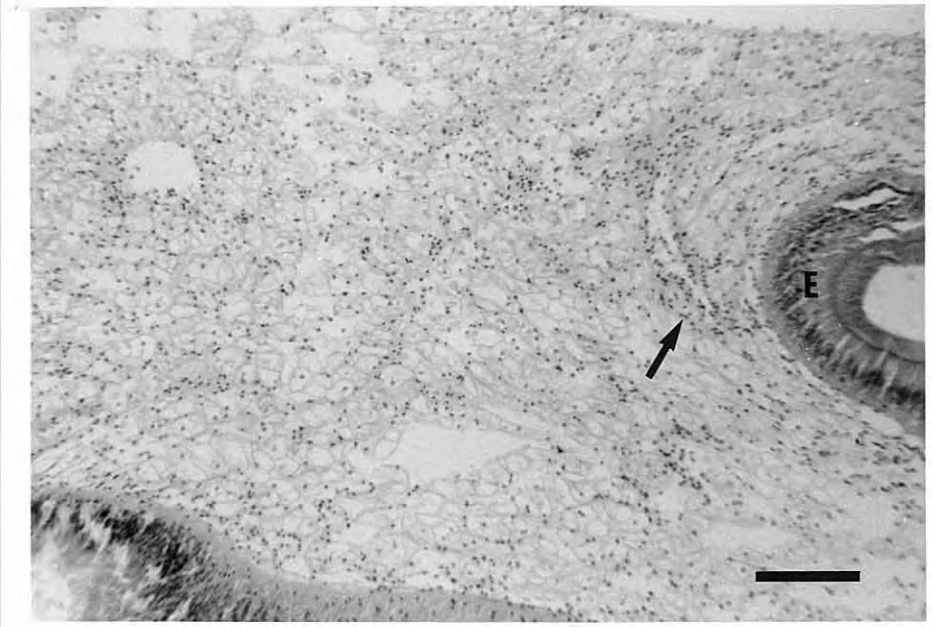


Fig 2.7: Section through connective tissue of *Crassostrea gigas* showing mild systemic infiltration of haemocytes scored as "HC 1D". Arrows show location of haemocytes, E = gut epithelium (H&E). Scale bar = 100 μm.

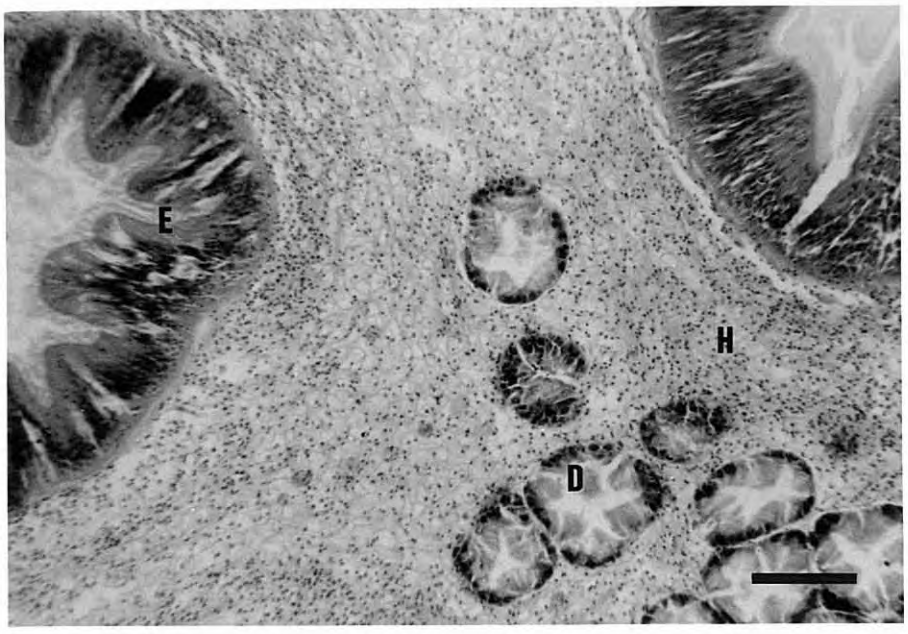


Fig 2.8: Section through connective tissue and gut showing moderate systemic infiltration of haemocytes scored as (HC 2D); H = location of haemocytes, E = epithelium of gut; D = digestive gland (H&E). Scale bar = 100 μm.

Haemocyte Infiltration

The degree of infiltration of haemocytes and its nature (localized (L) or diffuse (D)) was recorded. No infiltration, light, medium and heavy infiltration were recorded as 0, 1, 2, 3 respectively. Thus, an oyster with a moderate diffuse haemocytic infiltration was scored as HC 2D. Each score was recorded as diffuse or localized with localized infiltrations including peri-gut infiltrations, small patches of haemocytes and responses to invading organisms. An individual oyster may have had both localized and diffuse infiltration of haemocytes. Hence, when these scores are expressed as percentages, the total percentage is sometimes higher than 100%.

Figure 2.6 shows an oyster with a score of 0, figures 2.7 and 2.8 show haemocyte scores 1 diffuse and 2 diffuse respectively. No Pacific oysters were found with an infiltration score of 3 diffuse. Localised haemocytic infiltration stages 2 and 3 are shown in figures 2.9 and 2.10 respectively. Pacific oysters with a score of 1 localised were not seen.

Brown cells

Wandering or brown cells were scored on a scale of 0, 1 and 2 to describe low, moderate and high numbers of these types of cells respectively. The scores BWN 0, BWN 1 and BWN 2 are shown in figures 2.11, 2.12, and 2.13 respectively.

Digestive tubule epithelia height

A score of the height of the digestive tubule epithelia was made. A scale of 0, 1 and 2 were used to describe the three stages described by Couch (1985). Scoring followed the system where DTA (digestive tubule atrophy) 0 represented normal healthy digestive tubules (Fig 2.14), DTA 1 reduced height of digestive tubule epithelia (Fig 2.15) and DTA 2, this condition, combined with sloughing of cells into the lumen (Fig 2.16).

In an oyster section more than one of these stages may be exhibited in the digestive gland. Thus to obtain an accurate assessment of the state of the digestive gland overall, the percentage of digestive gland exhibiting each stage was recorded. For example, if all of the digestive tubules are at stage 0 then 100% of the digestive gland was recorded as "0". Alternatively, if portions of the digestive gland are at stage 2 then the record would read for example "stage 0 - 80%; stage 2 - 20%".

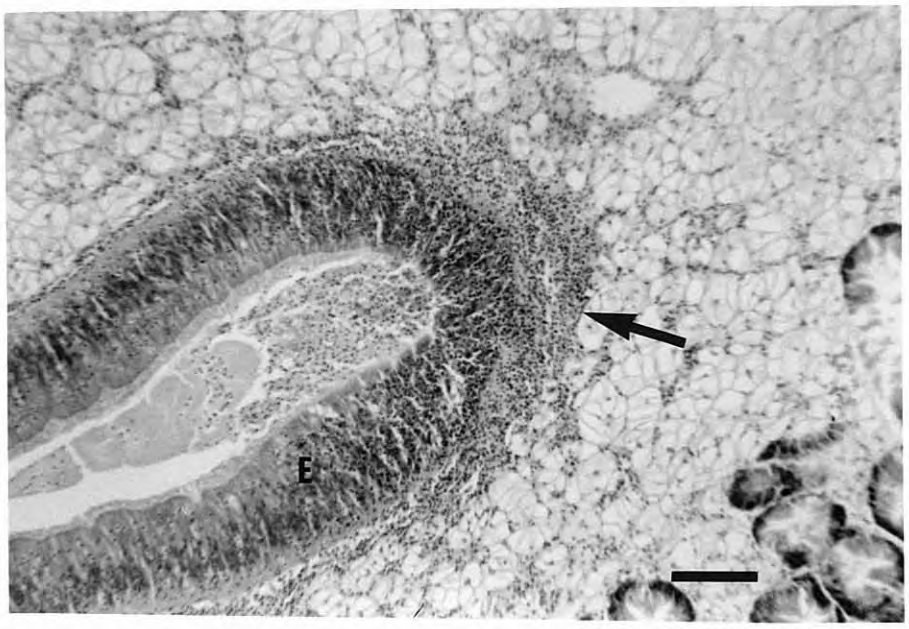


Fig 2.9: Connective tissue profile showing moderate focal haemocytic infiltration of the epithelium of the intestine and surrounding connective tissue - scored as "HC 2L". Arrows show location of haemocytes, E = intestine epithelium (H&E). Scale bar = 100 μm.

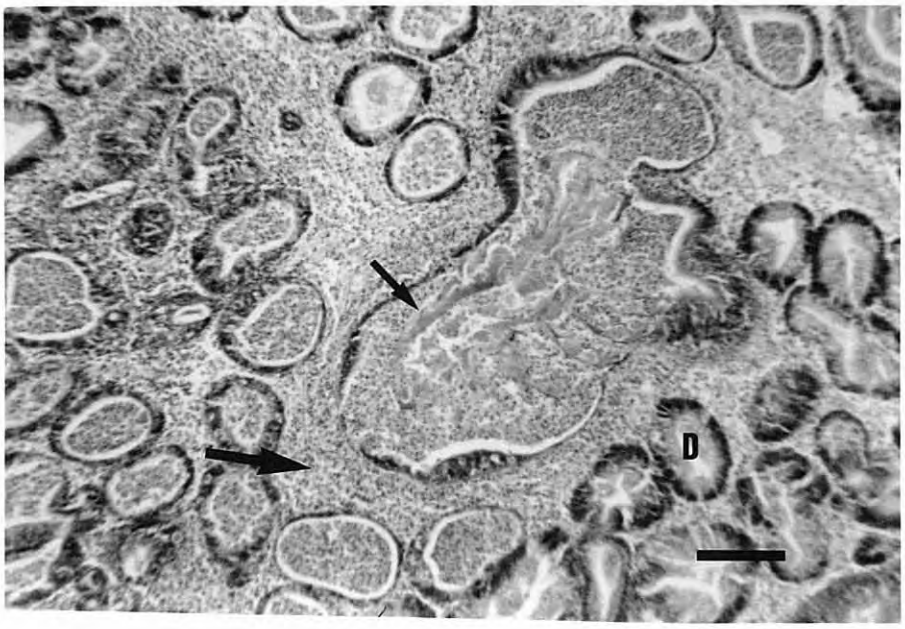


Fig 2.10: Connective tissue profile showing severe focal haemocytic infiltration of the digestive gland region - scored as "HC 3L". In this case the oyster is responding to the presence of a copepod, *Pseudomyicola spinosus* (small arrow). Large arrows show area of haemocytes, D = digestive gland (H&E). Scale bar = 100 μm.

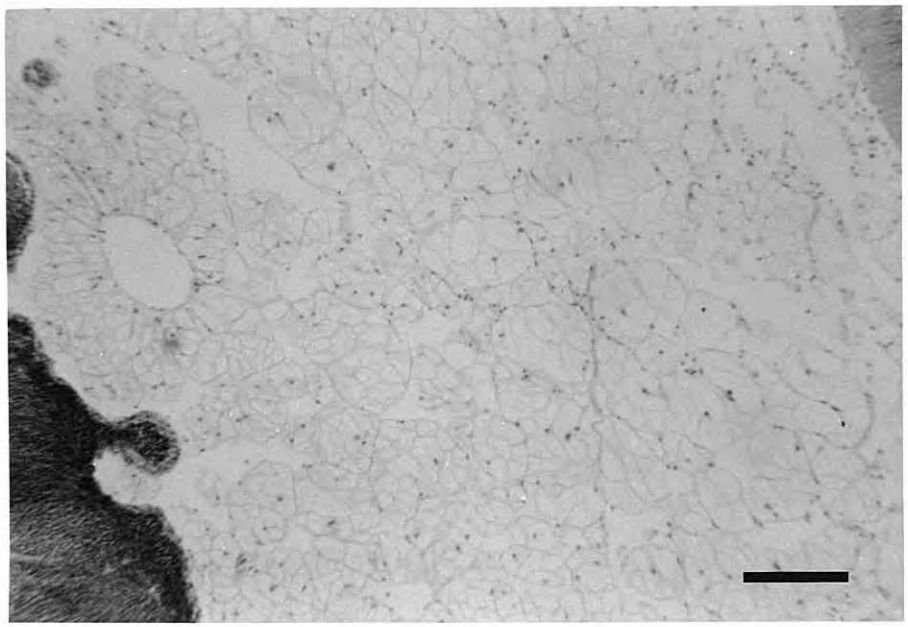


Fig 2.11: Connective tissue profile with minimal number of brown cells present scored as "BWN 0" (H&E). Scale bar = 100 μm.

Fig 2.12: Section through connective tissue and digestive gland showing the presence of moderate numbers of brown cells (arrows) in the connective tissue - scored as "BWN 1"; E = gut epithelium (H&E). Scale bar = 100 μm.

Fig 2.13: Section through connective tissue and gut epithelium showing large numbers of brown cells (arrows) in the connective tissue - scored as "BWN 2"; D = digestive gland, E = gut epithelium (H&E). Scale bar = 100 μm.

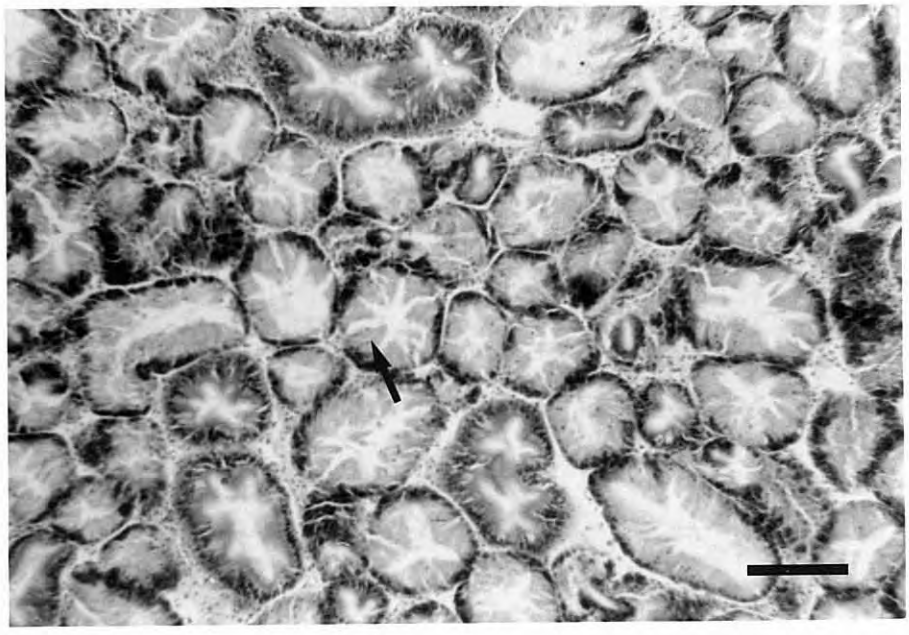


Fig 2.14: Profile of digestive tubules in *Crassostrea gigas* scored as "DTA 0". Note tall digestive epithelia (arrow) and tri and quadrate form of most tubules (H&E). Scale bar = 100 μm.

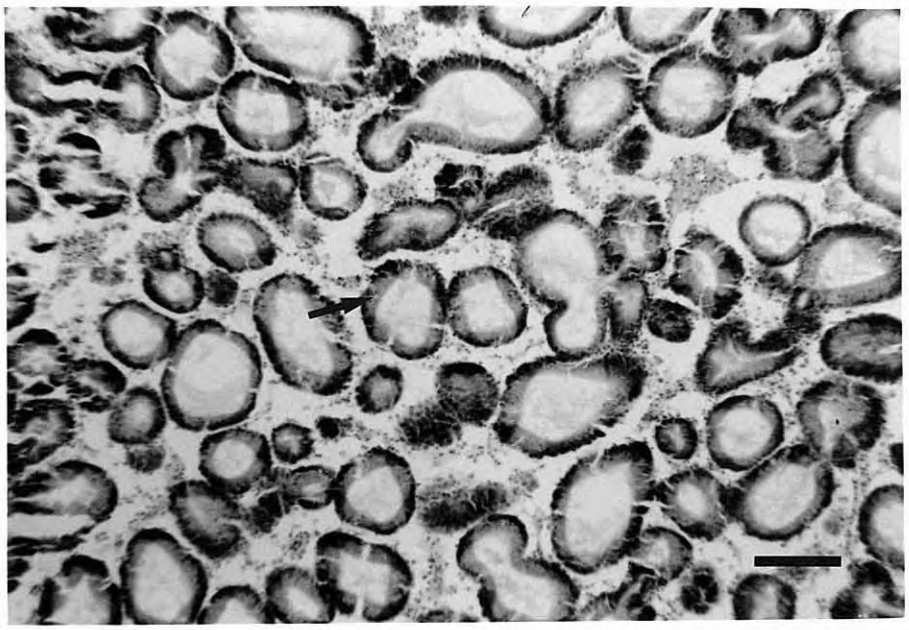


Fig 2.15: Section through digestive tubules scored as "DTA 1". Note the reduced height of digestive epithelium (arrows) and large lumen (H&E). Scale bar = 100 μm.

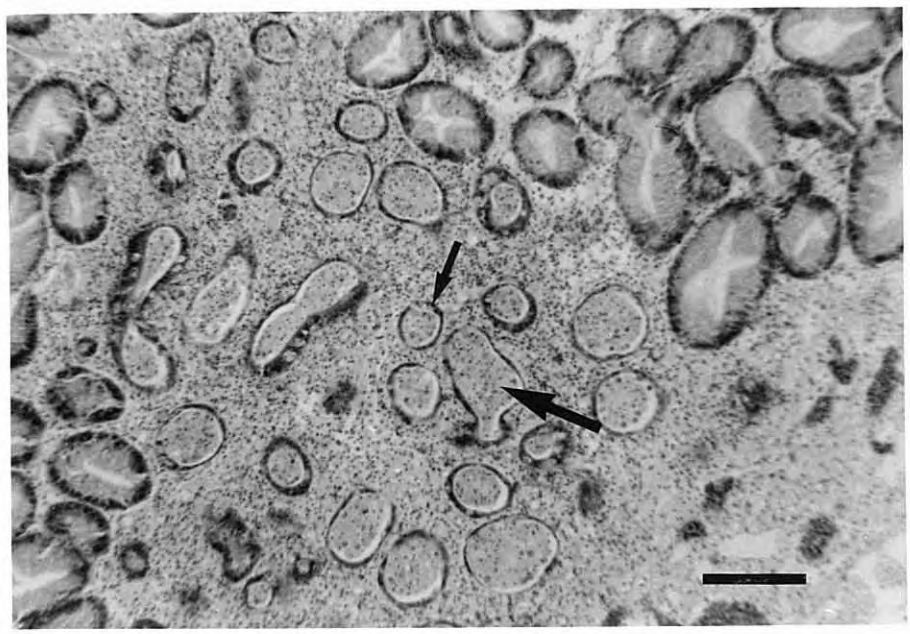


Fig 2.16: Section through digestive tubules scored as "DTA 2". Digestive epithelium is severely reduced and cells (small arrow) have sloughed into the lumen (large arrows) (H&E). Scale bar = 100 μm.

Gonad Index

Although the research on tissue changes centred on the non-reproductive tissues, the sex and approximate gonadal index was also recorded as changes in the histological appearance of tissues may vary during the gametogenic cycle. Gonads of Pacific oysters were staged using a variation of the system described by Dinamani (1974), used by Kent (pers. comm.).

The scoring system described the following stages.

Stage 1 Immature gonad of indeterminate sex.

Stage 2 Early developing gonad.

F2/M2 (Female 2/ Male 2)
Eggs with distinct nucleus, germ cells of both sexes apparent on only one side of follicle. Sex can be determined.

Stage 3 Developing gonad.

F3 Eggs are teardrop shaped and follicular channels or "curtains" are evident.

M3 The margin of the follicle is dark (basophilic) with sperm heads and the centre consists of sperm tails and is paler staining (eosinophilic).

Stage 4 Fully developed gonad/spawning.

F4 This stage is characterized by the appearance of large hexagonal, shaped eggs packed densly in the follicle. The cytoplasm is more basophilic than in earlier stages.

M4 Sperm are densly packed and the gonad is dark with no obvious lighter patches. At this stage the gonad takes on a swirled appearance.

Stage 5 Partially spent.

F5 Egg cells are large and are rounded compared with hexagonal in the previous stage. The gonad is "patchy" and haemocytes are present within the follicle.

M5 Sperm cells form a line around but slightly detached from the follicle edge giving a haloed appearance to the remaining gonad.

Stage 6 Mostly spent

FX/MX Only remnant gonad products remain. Follicles have partially collapsed and haemocytes are present within the follicles.

Stage 7 Regressed gonad.

R No remnant gonad product remains although the follicles have not completely closed (as in Stage 1).

All oysters collected in samples during the last 2 months of the study were scored by this method. For samples collected prior to this, a random sample of 10 oysters containing 5 male and 5 female oysters was selected from every sample and scored by this method.

2.4 Electron microscopy

Electron microscopy studies were conducted using glutaraldehyde-fixed and deparaffinized tissue. Glutaraldehyde was used where possible as a preferred fixative for preparation of tissue for electron microscopy. However, in some cases, the author was alerted to the presence of the organisms or lesions of interest only through examination of histological sections. Where possible, additional samples were obtained and samples were prepared for electron microscopy using glutaraldehyde. In many cases though, the organisms were rare or very localised in the tissue and the only option to obtain material for electron microscopy was to cut the area of interest from the appropriate wax block and follow procedures for deparaffinizing and preparation for electron microscopy.

In an attempt to study *Bonamia* sp parasites by electron microscopy, samples of wild *O. angasi* from a region known to be infected with *Bonamia* sp were collected on 1.7.92. Fifteen oysters with pale digestive glands or watery condition were prepared for electron microscopy.

Digestive gland tissue remaining after a section had been taken for histological examination was cut into $1mm^3$ blocks and fixed in 2.5% glutaraldehyde/ filtered seawater for 1-2 hours and placed in a buffer of filtered seawater overnight (pH 6.8) (M. Hine, pers comm). At both stages the samples were kept at 4°C. Tissues were then processed according to standard laboratory procedures for electron microscopy detailed as follows.

The seawater buffer was removed and 1% osmium tetroxide in 0.1 M sodium cacodylate buffer (pH 6.8-7.3) was added for one hour at room temperature. Enough osmium tetroxide was added so that the tissues were covered. Osmium tetroxide was removed and the tissues rinsed in distiled water. Prestaining was

achieved by placing the tissue in 5% uranyl acetate (saturated) for 30 minutes. Tissues were dehydrated through a series of alcohols of 50, 75 and 95% with two changes of 10 minutes each. At this stage tissue can be stored indefinitely at room temperature in 95% alcohol.

Tissues were embedded in resin prior to sectioning by the following method. The epon (Ladd LX112) was prepared by mixing for 1 hour before adding tri (dimethyl amino methyl) phenol (DMP 30) and mixing for a further 30 minutes. Tissue was dehydrated in 100% alcohol (2 changes of 20 minutes each) and cleared in 100% propylene oxide (2 changes of 20 minutes each). Tissue was then transferred to a mixture of 50% epon / 50% propylene oxide for 2-3 hours before infiltrating in a pure epon mixture on a rotator for a further 2-3 hours. Pure fresh epon was added to the capsules and the tissue transferred. Capsules were held in a 37°C oven for 12-24 hours then at 60°C for a further 36 hours.

Electron microscopy studies of rickettsiales inclusions were conducted on deparaffinized tissue of 2 x *C. gigas* collected from the east coast and the northwest on 3.10.90 and 11.10.90 respectively, and 2 x *O. angasi* collected from the south east on 12.2.91. Papovavirus inclusions in 6 x *C. gigas* collected from D'Entrecasteaux Channel on 20.11.92 were also examined ultrastructurally using the same technique.

Methods of Hayat (1970) were followed for deparaffinizing wax blocks for electron microscopy. This involved cutting relevant tissue from the wax block into pieces of $3mm^3$. These were deparaffinized by placing in xylene for 1 hour on a rotator. Tissue was rehydrated in a series of alcohols for 15 minutes in each of 100, 95, 75, 50, and 15% alcohol, and left in a cacodylate buffer overnight. The remaining tissue was retrimmed to $1mm^3$ pieces and post fixed for 1 hour in OsO_4. Standard laboratory procedures (op. cit.) were then followed to complete processing.

An additional collection of *O. angasi* was taken on 22.10.92 from the lease in the south east from which *O. angasi* has previously been collected for electron microscopy studies. Earlier testing showed this population had a relatively high prevalence of rickettsiales inclusions and so was selected for further electron microscopy studies. The sample consisted of fifteen oysters all of which were dissected and prepared for histological examination. Of these, ten were selected for electron microscopy preparation by dissecting a portion of the digestive gland

remaining after a section was taken for Davidson's fixation. $1mm^3$ pieces of tissue were fixed in 4% glutaraldehyde for 1-2 hours. Again standard laboratory procedures were followed for preparation electron microscopy.

2.5 Perkinsus testing

During both summer periods covered by the sampling period ie Dec 1990 - Feb 1991 and Jan 1992 - Feb 1992, 488 oysters were tested for the presence of *Perkinsus spp* using a modification of the Ray technique described by Quick (1972).

A small piece of tissue was dissected from the oyster meat, either rectum and gill or digestive gland and labial palps. This was placed in a bottle containing fluid thioglycollate media* (FTM) and antibiotics and stored in the dark at room temperature for at least 4 days. After the period of culture, the tissue was removed from the culture vial, placed on a clean petri dish and a couple of drops of Lugols iodine* added. After approximately 5 minutes the tissue was teased apart and examined under the dissecting microscope for signs of the characteristic sporulating stages.

* A measure of 29.3g of FTM (oxoid) was rehydrated per 1L of distilled water containing 20g NaCl. The media was dispensed in 10mL aliquots into McCartney bottles, autoclaved, then stored in the dark at room temperature until needed. Just prior to use 0.07mL of antibiotic mixture was added to each 10mL aliquot of media to prevent decomposition of tissue by bacterial invasion. The antibiotic mixture was composed of 500000µg chloramphenicol and 500000 units mycostatin in 16.6 mL distilled water. Chloramphenicol was first dissolved in 0.5-1.0 mL ethanol before adding to water.

Lugols iodine was also prepared just prior to use. 10g of potassium iodide was dissolved in 100mL of distilled water to which was added 1g of iodine crystals. This solution was then stored in a dark bottle away from light.

2.6 Environmental data

Information on temperature and salinity at oyster leases was collected by myself or staff from the Division of Sea Fisheries (Tasmanian Shellfish Quality Assurance Program). Out of a total of 58 samples collected, this information was collected at the same time as oyster samples on 26 occasions.

Salinity and temperature measurements were made with a salinity/temperature meter (WTW Conduktometer LF 191) or by use of a hydrometer and mercury

thermometer respectively. Where a hydrometer was used, the specific gravity of the water was converted to a measure of salinity using standard conversion tables.

2.7 Mortality information

During this survey, selected growers from each growing area were requested to contribute farm records showing data on mortality or poor growth. Additional mortality information was gathered through on farm visits or information provided by staff of the Quality Assurance Program or was volunteered by growers.

2.8 Statistical Analysis

2.8.1 Temporal, geographic and host age distribution of parasites.

Differences in the prevalence of each parasite, commensal or abnormal condition were analyzed using a generalized linear model. The model used in this analysis assumed that the number of successes (counts of occurrences of each parasite/commensal/condition per number of oysters examined) were distributed binomially. A logit link function was used to fit the models. The models were developed on the characteristics (variates) of age, area and season for each species of oyster. The software program used in the analysis was GENSTAT (IBM compatible).

For some factors (ovacystis, Virus X, *Bonamia* sp, gill ciliates A and B and turbellarians) there were insufficient observations to conduct such analysis. Where appropriate, these factors were analysed by other methods described later.

For factors where the number of observations was sufficient, the most complex model with highest order interactions i.e. age x area x season was first fitted to the data and by comparison of more complex models to less complex models, an appropriate model was obtained. This was achieved by the following methods. Once the most complex model had been fitted, the residual mean deviance (r.m.d) and the associated degrees of freedom (d.f.) were recorded. The next simplest model obtained by dropping one variate was fitted and the r.m.d and d.f. again recorded. To determine whether the variate should be dropped, it was assumed that the change in r.m.d. between the two models was distributed as a Chi-square

with the degrees of freedom equal to the changes in the residual d.f. between the two models i.e.

Residual mean differences = (Simple model r.m.d.) - (Complex model r.m.d.)
and
degrees of freedom = (Simple model residual d.f) - (Complex model residual d.f.).

Where it was possible to drop more than one interaction (eg area x age, area x season, age x season) each interaction was dropped in turn whilst including the other two interactions and then each model was tested individually. The significance of all models was compared and the interaction in the model with the least significance was dropped. The remaining models were again tested until none of the models contained the remaining variates/interactions could be deleted at a 5% level of significance. (If none of the variates were significant the overall mean percentages were compared).

Once the model had been fitted, t-tests generated by GENSTAT were used to determine where the differences lay.

2.8.2 Comparison of prevalences of *Bonamia* sp in *O. angasi* stocks

X^2-tests were used to determine if prevalences of *Bonamia* sp in *O. angasi* at different sites or where oysters were from different sources, were significantly different at $p<0.05$. Where proportions were significantly different, the following equation was used to determine where the differences lay.

$$p_i - p_j > (X^2)^{0.5} [p(1-p)(1/n_i + 1/n_j)]^{0.5}$$

where $p_i = \frac{r_i}{n_i}$ and $p_j = \frac{r_j}{n_j}$

where r_i = no with *Bonamia* in sample i
and n_i = no of oysters in sample i

and where:

$$p = \frac{r_i}{n_i} = \frac{r_1+r_2+r_3}{n_1+n_2+n_3}$$ where r_i=no. with *Bonamia* n_i=no. in sample

if this equation is true then proportions being tested are significantly different at the chosen level of significance for the X^2 statistic.

T-tests were not used as the data was in the form of counts (non-continuous data).

2.8.3 Measurements of organisms

Measurements of parasites or commensal organisms were expressed as a mean value with 95% confidence intervals as the values were normally distributed.

2.8.4 Change in histological appearance over time and between areas.

Data on changes of histological appearance of tissue including leydig tissue, haemocytic infiltration, number of brown cells in Pacific oysters were analysed by area and over time using a Chi - square test. Where there were low numbers in a category (eg LEY 1+2; HC 2+3L; HC 1+2D; BWN 1+2) the two categories were pooled. Using this method, the relative proportions of each score were compared with expected values generated from a contingency table. 3x4 contingency tables were used for analysis by area and 2 x 16 contingency tables were used for analysis by monthly samples.

Where the resultant X^2 figure was significant ($p<0.05$) this represented significant differences in relative proportions of tissue scores between areas or seasons. Where X^2 values of individual cells in contingency tables were large (>10) this represented major contributions to the overall result of significant differences between areas or seasons. The data was then examined to see whether these cells represented unusually high or low values of that category. The patterns of unusually high or low values for a particular score of tissue condition were determined for each month in each area.

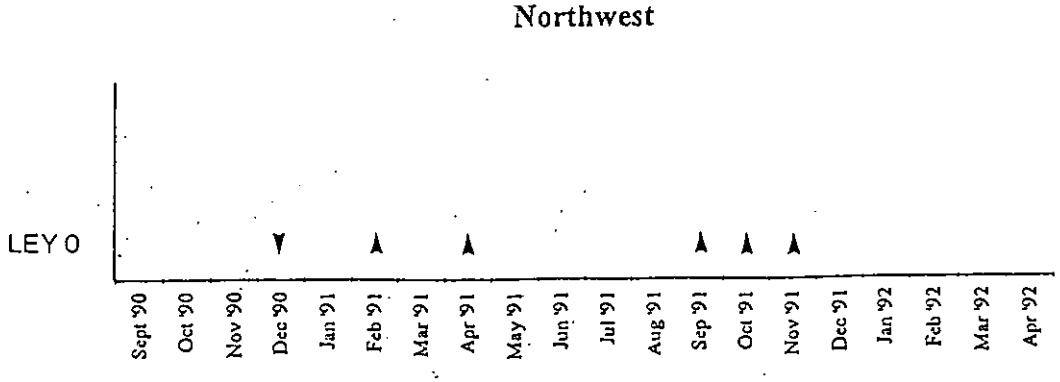
For example:

The relative proportions of connective tissue edema (LEY 1, LEY 1+2) were significantly different between months over the period of sampling in Area 1. Overall X^2 result = 319.17 ($P<0.05$) (see X^2 result below).

	LEY 0		LEY 1+2	
Oct 90	0.12	+	0.17	
Dec 90	**43.00**	+	**63.76**	
Jan 91	1.34	+	1.99	
Feb 91	**12.27**	+	**18.19**	
Apr 91	9.57	+	**14.19**	
May 91	0.08	+	0.12	
Jun 91	0.002	+	0.003	
Jul 91	2.07	+	3.07	
Sep 91	**12.00**	+	**17.79**	
Oct 91	**22.73**	+	**33.70**	
Nov 91	**11.35**	+	**16.83**	
Jan 92	3.85	+	5.70	
Feb 92	0.07	+	0.12	
Mar 92	4.21	+	6.26	
Apr 92	5.88	+	8.72	= 319.17 ($p<0.05$)

Large contributions to the significant overall X^2 value (bold type) were made by the individual cells LEY 0, LEY 1+2 in December 1990, February, September, October and November 1991. At other times of the year, relative proportions did not contribute as strongly to the overall X^2 value. By examination of the data it was found that the number of oysters with a score of LEY 0 was low during December 1990 and was high for the months February, September, October and November 1991.

Hence this trend is represented graphically as follows:-



2.8.5 Correlation of histological appearance and environmental variables.

To determine whether the changes in histological appearance of Pacific oysters was influenced by environmental variables or stage of gametogenesis, correlation analysis between these factors and temperature and salinity data and proportion of post spawned oysters was performed using the computer program EXCEL 4.0.

CHAPTER 3. RESULTS

This chapter is divided into three sections. In the first, quantitative data on the prevalence of each organism in each species of oyster is described. Generalized linear models which best fitted the data for each type of organism are given where available, and from these, trends in the geographic and seasonal distribution are described. Significant differences between areas or seasons were determined at the 95% level of significance. Qualitative data including morphological descriptions, site and intensity of infection, associated pathology and effect on the host has been placed in the second section.

The third section reports the findings of variations in histological appearance of Pacific oysters, the seasonal patterns in these changes and the influence of environmental factors.

3.1 QUANTITATIVE RESULTS

The prevalences, geographic distribution and location in the tissue of parasites and commensals or abnormal conditions associated with *C. gigas* and *O. angasi* are listed in Tables 3.1 and 3.2, respectively.

Eight types of commensal or parasitic organisms were associated with *C. gigas* including a viral infection of the gametes, rickettsial inclusions, two species of ciliate, two protozoans of unknown taxonomy, a turbellarian, spionid polychaetes and two types of copepod. A small number of oysters were also observed with watery or emaciated condition for which no apparent cause was detected.

O. angasi were infected with three types of commensal organisms including rickettsial inclusions, one species of ciliate, and two types of copepod. In addition, flat oysters were infected with a serious pathogen (*Bonamia* sp) and a viral infection of unknown significance.

The resultant generalised linear models which fitted the data for each type of commensal or parasite for each species of oyster are given in Table 3.3. The data for ovacystis, Virus X, gill ciliates, gill copepods and turbellarians had insufficient observations for analysis by this method and are not included in this table.

Table 3.1: Prevalence and distribution of abnormal conditions and commensals and parasites of *Crassostrea gigas*.

Commensal	prevalence (%)	Area	Location in tissue
Ovacystis	<1.0	1,2,3,4	ova nuclei
Abnormal ova	3.5	1,2,3,4	
Rickettsial inclusions	**1.4**	1,2,3,4	
Type A_1	0.5		digestive gland
Type A_2	0.7		digestive gland
Type B	0.2		digestive gland
Type C	(1)*		digestive gland
Type D	(2)*		stomach
Type E	(2)*		connective tissue
Ancistrocoma sp	13.5	1,2,3,4	digestive gland
Trichodina sp	3.5	1,2,3,4	gill
Gill ciliate A (*Sphenophyra* sp ?)	<1.0	1,2,3,4	gill
Gill ciliate B	<1.0	1,2,3,4	gill
Turbellarian	(5)*	1,2,3	intestine, digestive gland
Pseudomyicola sp	3.2	1,2,3,4	digestive gland
Gill copepod	0.1	1,2	gill
Watery	2.5	1,2,3,4	

*values in parentheses are counts

Table 3.2: Prevalence and distribution of abnormal conditions, commensals and parasites of *Ostrea angasi*.

Commensal	Prevalence (%)	Area	Location in tissue
Virus X	(1)*	2	connective tissue
Rickettsial	11.1	1,2,3,4	digestive gland
Bonamia sp	3 - 22	2,4^	digestive gland, gills
Ancistrocoma sp	6.0	1,2,3,4	digestive gland
Pseudomyicola sp	3.7	1,2,3,4	digestive gland
Gill copepod	(1)*	3	gill
Watery	14.0	2,3,4	

*values in parenthesis are counts
^oysters from this lease have since been destroyed.

Viruses

Papovavirus

Ovacystis infection, caused by a papovavirus, was found in 24 Pacific oysters (0.45%) and was not found in flat oysters. Infections were found predominantly in females with only two males infected. This condition was recorded from Pacific oysters collected in all growing areas.

A small proportion of ova of an additional 3.5% of Pacific oysters had opaque, darkly staining nuclei, which, as will be discussed later, may represent an early ovacystis infection.

Virus X

Intranuclear inclusions were found in the connective tissue of one flat oyster collected from the east coast on 20.2.92. Identical inclusions seen in wild populations of flat oysters have been examined by electron microscopy and contained herpes-like viral particles (Handlinger, pers comm).

Table 3.3: Resultant Generalised Linear Models for Data on Prevalence of Organisms in *Crassostrea gigas* and *Ostrea angasi* in Tasmania.

Species	Parasite type	Generalised linear model
C. gigas	Rickettsiales	Age + Area + Season + Age x Area + Area x Season
	Ancistrocoma sp	Area + Season + Area x Season
	Trichodina sp	Age + Season + Area + Age x Season + Area x Age + Season x Age
	Shellblisters	Age + Season + Area + Age x Season + Age x Area + Season x Area + Age x Season x Area
	Pseudomyicola sp	Age + Area + Season + Area x Season
	Watery	Age + Area + Season + Area x Season
O. angasi	Rickettsiales	Area + Season + AreaxSeason
	Bonamia sp	Age + Season
	Ancistrocoma sp	Area
	Shellblisters	Age + Area + Season + AgexSeason + AreaxSeason
	Pseudomyicola sp	Age + Area
	Watery	Age + Season + Age x Season

Rickettsial inclusions

Rickettsial inclusions were observed in 1.4% of Pacific oysters from all growing areas. Figure 3.1 shows the prevalence of these organisms over the study period for the four areas studied. The generalised linear model (GLM) determined that the prevalence of rickettsial inclusion in Pacific oysters was significantly influenced by the age of the host, area and season and the interactions of age x area and area x season. Examination of stepwise comparisons between each category showed that the prevalence of inclusions was significantly higher on the east

Fig 3.1 : Variation in prevalence of rickettsial inclusions in Crassostrea gigas over time.

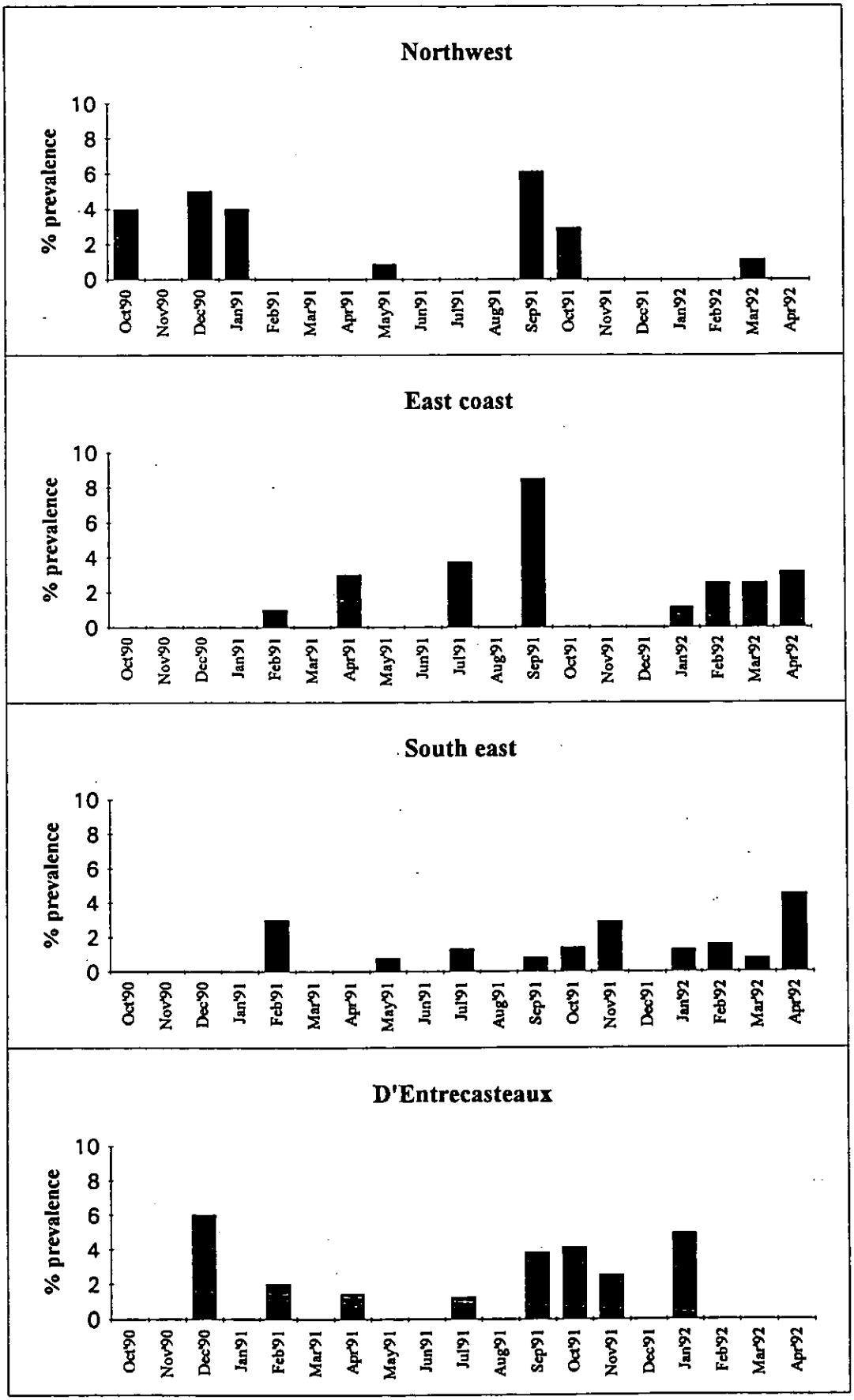
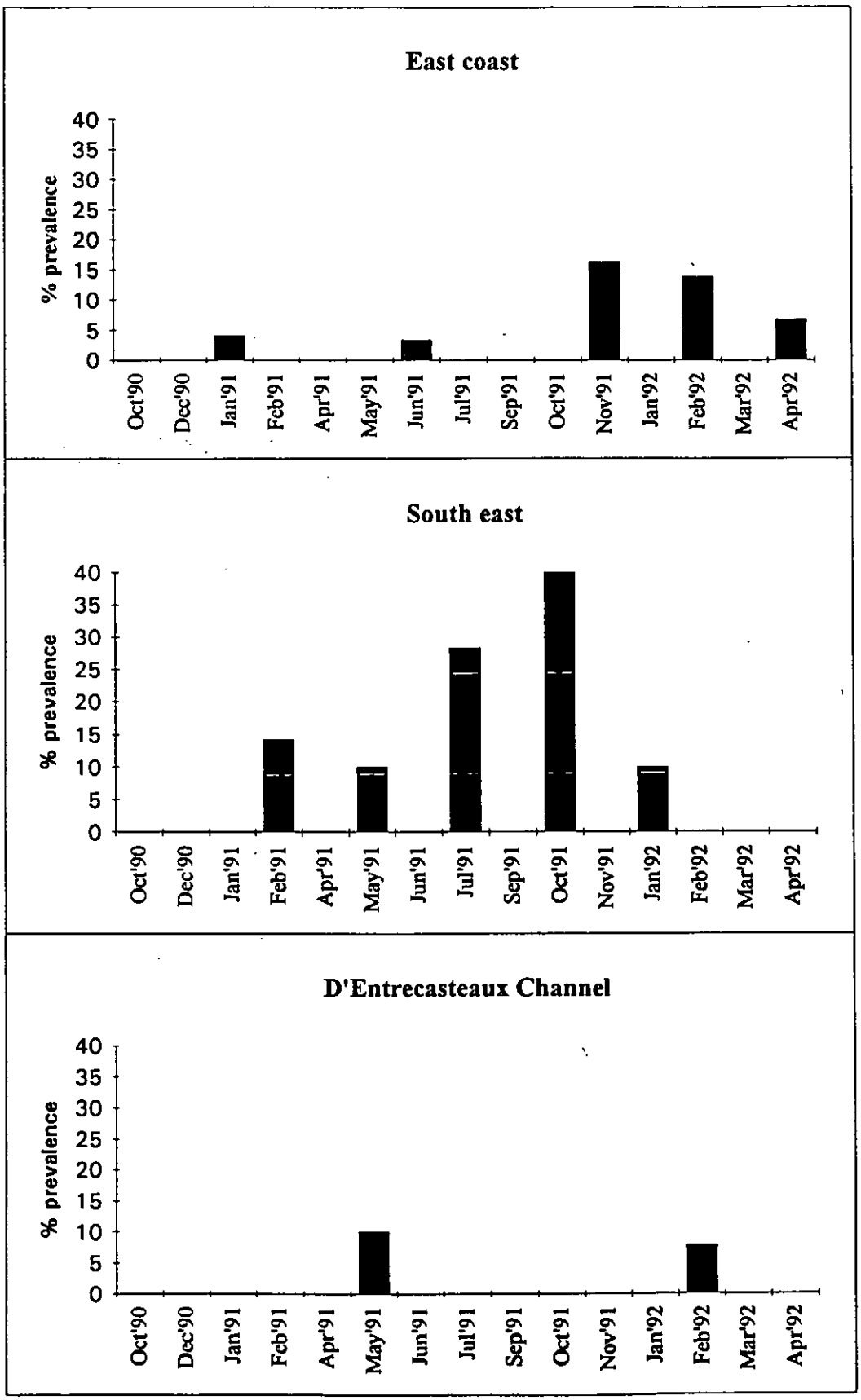


Fig 3.2: Variation in prevalence of rickettsial inclusions in Ostrea angasi over time*.



*Not present in samples from the north west.

coast during winter than in any other area or at any other time of the year.

Six morphological types of amorphous basophilic inclusions were found in the tissue of Pacific oysters at the light microscope level. Examination by electron microscopy of some of these inclusions revealed they were comprised of rickettsial organisms similar to those described in the literature from other species of shellfish.

Rickettsial inclusions were noted in 11.1.% of flat oysters. Prevalence of inclusions in each of the four areas is shown in Fig 3.2. The GLM showed that prevalence of infection was influenced by area and season and the interaction between area and season. However, there were no significant differences between any two categories as determined by stepwise t-tests. This apparent discrepancy in analysis may result from low sample sizes in each category being compared. Thus, the general trends determined using the model may not be apparent as significant differences between individual cells. From Fig 3.2, prevalence of rickettsial inclusions in flat oysters was highest on the south east coast.

Protozoa

Perkinsus spp

All 488 cultures for *Perkinsus spp* were negative.

Bonamia sp

The protozoan parasite *Bonamia* sp was found in stocks of cultivated *O. angasi* at a prevalence of between 3 and 22%. *Bonamia* sp was first detected by Dr Judith Handlinger in February 1992, in samples of wild oysters from Georges Bay on the east coast of Tasmania (Handlinger, pers. comm.). Subsequently, all samples of flat oysters collected during this survey from farms in areas known to be infected with *Bonamia* sp were re-examined. From areas where *Bonamia* sp had not been found in wild populations of *O. angasi*, samples collected in summer and autumn of previous years were re-examined. The results of these retrospective studies are shown in Table 3.4. The results of examination of the extraordinary samples collected on 14.4.92 from Georges Bay are shown in Table 3.5.

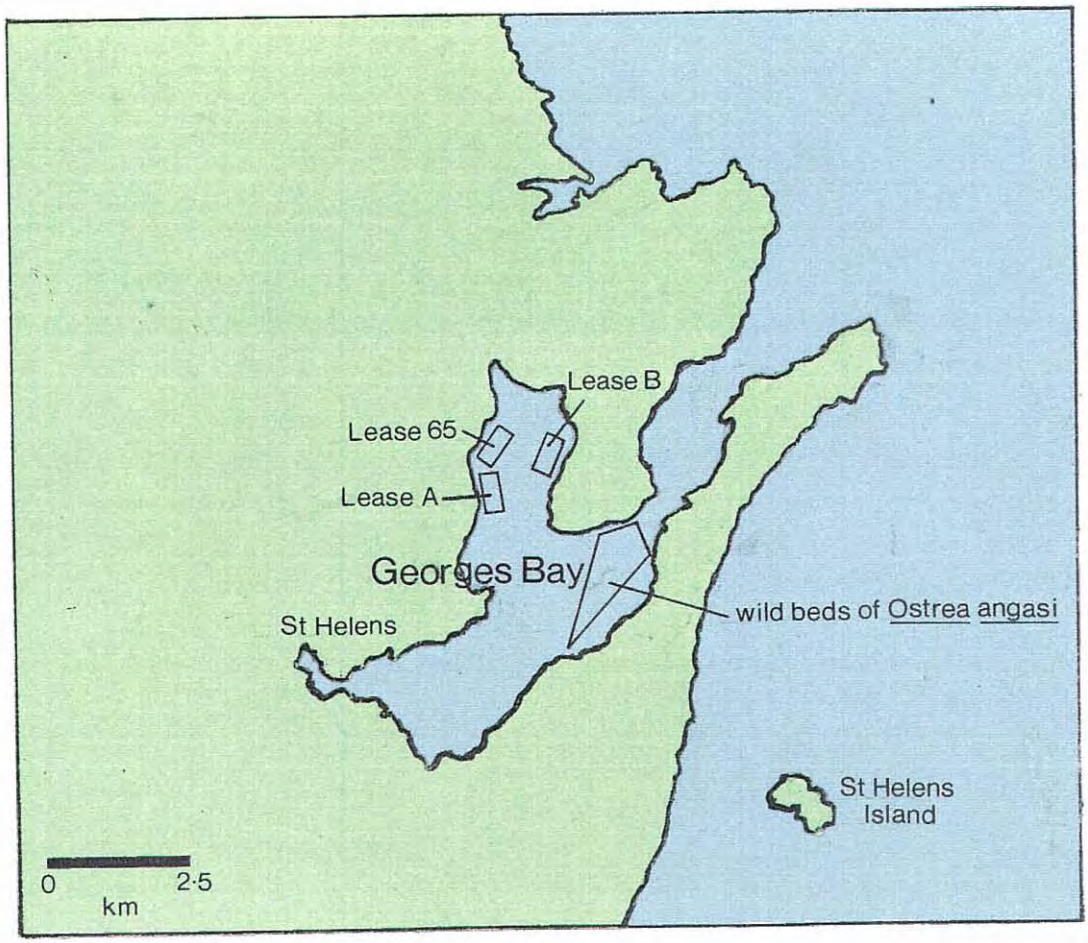


Fig 3.3: Map of Georges Bay showing oyster leases and wild beds of *Ostrea angasi* infected with *Bonamia* sp.

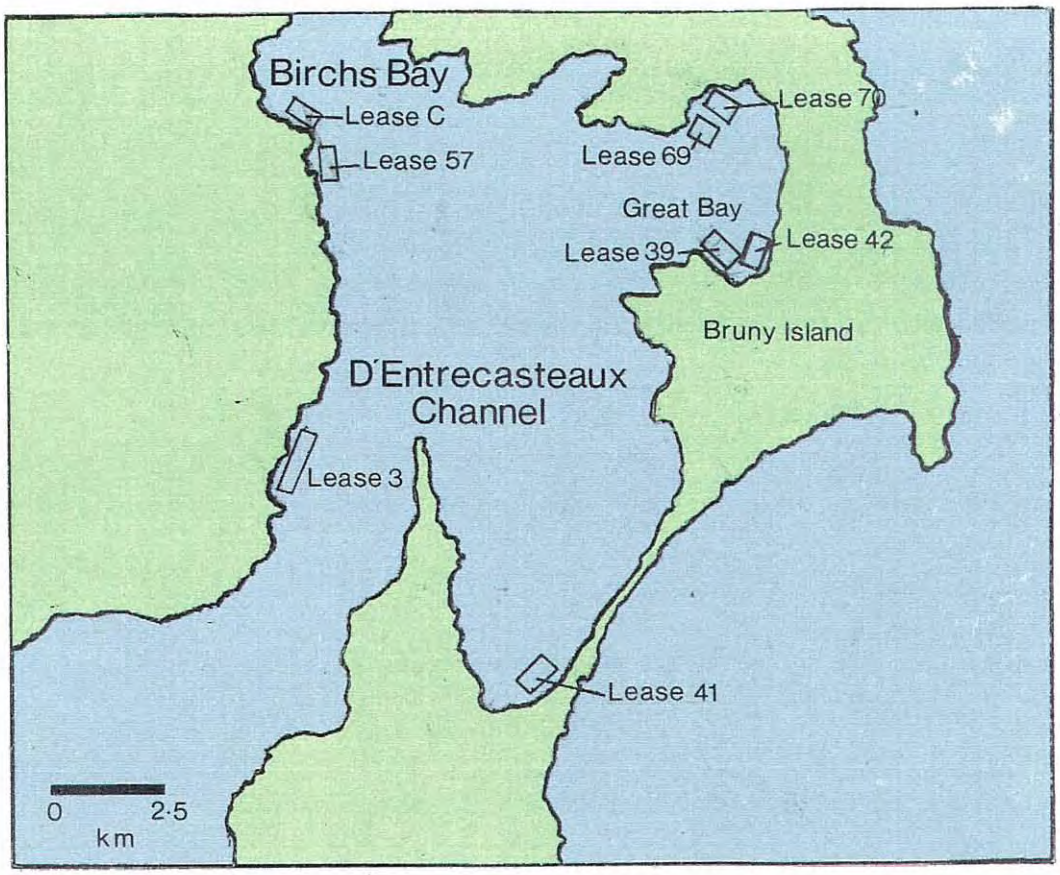


Fig 3.4: Map of northern region of the D'Entrecasteaux Channel showing oyster leases including Lease C in Birchs Bay which held *Bonamia* sp infected *O. angasi*.

Farms in two bays in Tasmania; Georges Bay on the east coast (Fig 3.3) and Birchs Bay in D'Entrecasteaux Channel (Fig 3.4), were found to be infected. Retrospective examinations showed that two leases (termed lease A and B) in Georges Bay were infected. Prevalence of infection in wild harvested oysters was not significantly different between these two leases at any time of sampling. It is interesting to note however, that although prevalence of *Bonamia* sp was slightly higher in samples from lease B, anecdotal reports of mortality were much lower (Table 3.5).

On lease A, samples collected in January 1991, July 1991 and April, 1992 were infected at a prevalence of 33.0, 8.1 and 23.7% respectively. In the sample collected in April, 1992 there was no significant difference ($p>0.05$) in the prevalence of *Bonamia* sp between groups of oysters reportedly experiencing different mortality rates (20 and 40%).

Bonamia sp was found in samples collected from lease B in January 1991, November 1991 and April 1992 at prevalences of 10, 5 and 35% respectively. The prevalence of *Bonamia* sp in April in wild harvested oysters (35%) placed on this lease, was significantly higher ($p<0.05$) than in naturally set oysters (10%) growing adjacent to this stock.

A total of 84 flat oysters were submitted from a lease in Birchs Bay (Lease C) during the period 4.9.90 - 19.2.92 (Table 3.4). *Bonamia* sp was only detected in a sample collected on 19.2.92 at a prevalence of 8.3%. This lease had received shipments of oysters from infected beds in Georges Bay since 14.6.90 as part of experimental studies by Division of Sea Fisheries, Tasmania. All *O. angasi* on this lease have since been removed and destroyed.

From the GLM, age of host and season were factors which significantly influenced the pattern of infection of *O. angasi* by *Bonamia* sp.

Due to the inconsistent nature of the sampling of *O. angasi* at these infected sites, a seasonal pattern of prevalence of *Bonamia* sp was not obtained. However, highest prevalence in Georges Bay (33 and 35%) was seen in samples taken in January 1991 and April 1992, respectively.

Table 3.4: Results of Initial and Re-examination of *Ostrea angasi* for the Presence of *Bonamia* sp.

Date	Sample	Location	Initial	Re-exam.
4.9.90	50x 4 year	Birch's Bay	-ve	-ve
6.12.90	8x 30mm	Pittwater	-ve	-ve
	10x 2 year	Pittwater	–ve	-ve
29.1.91	10x 50mm	Georges Bay	-ve	+ve (1/10)
	10x 90mm	Georges Bay	-ve	+ve (1/10)
31.1.91	12x 60mm	Georges Bay	-ve	+ve (4/12)
12.2.91	10x 1 year	Blackman Bay	-ve	-ve
	15x 3-4 year	Blackman Bay	-ve	-ve
13.2.91	12x 50mm	Hastings Bay	-ve	-ve
14.5.91	5x 1-2 year	Birch's Bay	-ve	-ve
	5x 3-4 year	Birch's Bay	-ve	-ve
2.7.91	10x 1-2 year	Georges Bay	-ve	+ve (1/9)
17.7.91	12x 0-1 year	Georges Bay	-ve	-ve
17.7.91	10x 1-2 year	Georges Bay	-ve	-ve
16.11.91	12x 1 year	Duck Bay	-ve	-ve
	12x 2 year	Duck Bay	-ve	-ve
27.11.91	13x 2 year	Georges Bay	-ve	-ve
	20x 1 year	Georges Bay	-ve	-ve
	20x 2-3 year	Georges Bay	-ve	+ve (2/19)
20.1.92	10x 3 year	Eaglehawk Neck	-ve	-ve
20.2.92	10x 2-3 year	Ex Eaglehawk Neck	-ve	-ve
	20x 1 year	Great Swanport	-ve	-ve
	20x 2-3 year	Great Swanport	?	-ve

-ve = negative for the presence of *Bonamia* spp
+ve = positive for the presence of *Bonamia* spp

Table 3.5: Results of examination of *O. angasi* for *Bonamia* sp in an extraordinary sample collected from Georges Bay, Tasmania on 14.4.92.

Sample	n	Age	Est. Mort	Source	Result/ prevalence (%)	
A1	30	>2yrs	20%	wild beds	7/30	23.3%
2	30	>2yrs	40%	wild beds	8/29	27.6%
B1	20	<2yrs	15%	wild beds	5/20	25.0%
2	40	>2yrs	15%	wild beds	16/40	40.0%
3	60	>2yrs	?	natural set	6/60	10.0%

Ancistrocoma sp

The ciliate, *Ancistrocoma* sp was found in 13.5% of Pacific oysters and 6.0% of flat oysters and was recorded from all growing areas.

Prevalence of the parasites in Pacific oysters in each of the four areas over the period of study is shown in Fig 3.5. In Pacific oysters, the pattern of prevalence was significantly influenced by area and season and their interaction (area x season). Specifically, during winter and spring prevalence was significantly higher in the channel and southeast regions than the east coast and the northwest.

In the south east region, numbers were significantly higher in winter and spring than in summer and autumn. On the east coast the prevalence in summer was significantly higher than at any other time of the year. In other areas the prevalence was not significantly different between seasons.

Ancistrocoma sp was found in flat oysters in all growing areas. The prevalence of *Ancistrocoma* sp in flat oysters was determined only by area, with infection significantly higher in the southeast than on the east coast.

Trichodina sp

Ciliates resembling *Trichodina* sp were apparent on the gills and interlamellar epithelium of Pacific oysters in all areas with an average prevalence of 3.5%.

Fig 3.5: Variation in prevalence of Ancistrocoma sp in Crassostrea gigas over time.

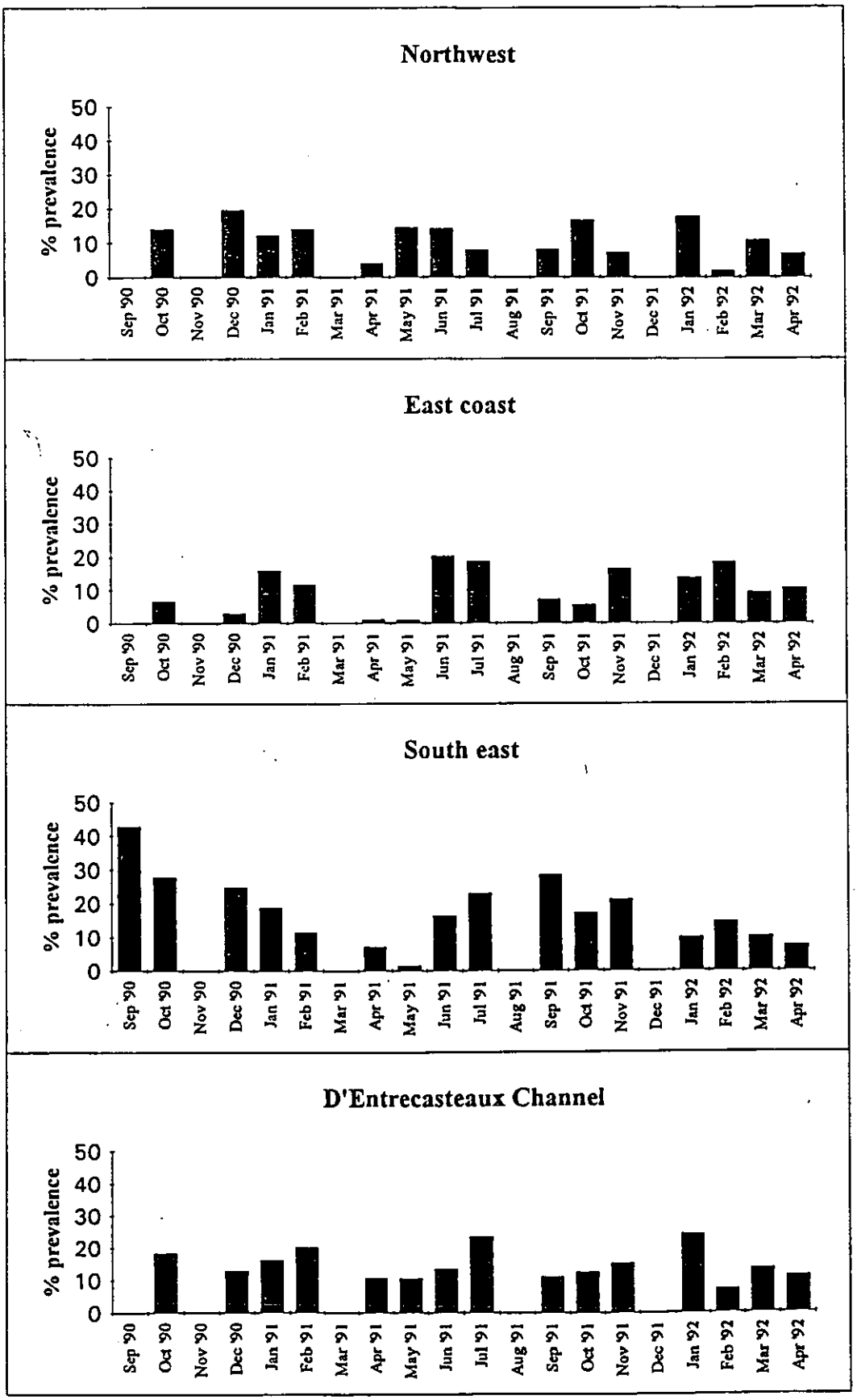
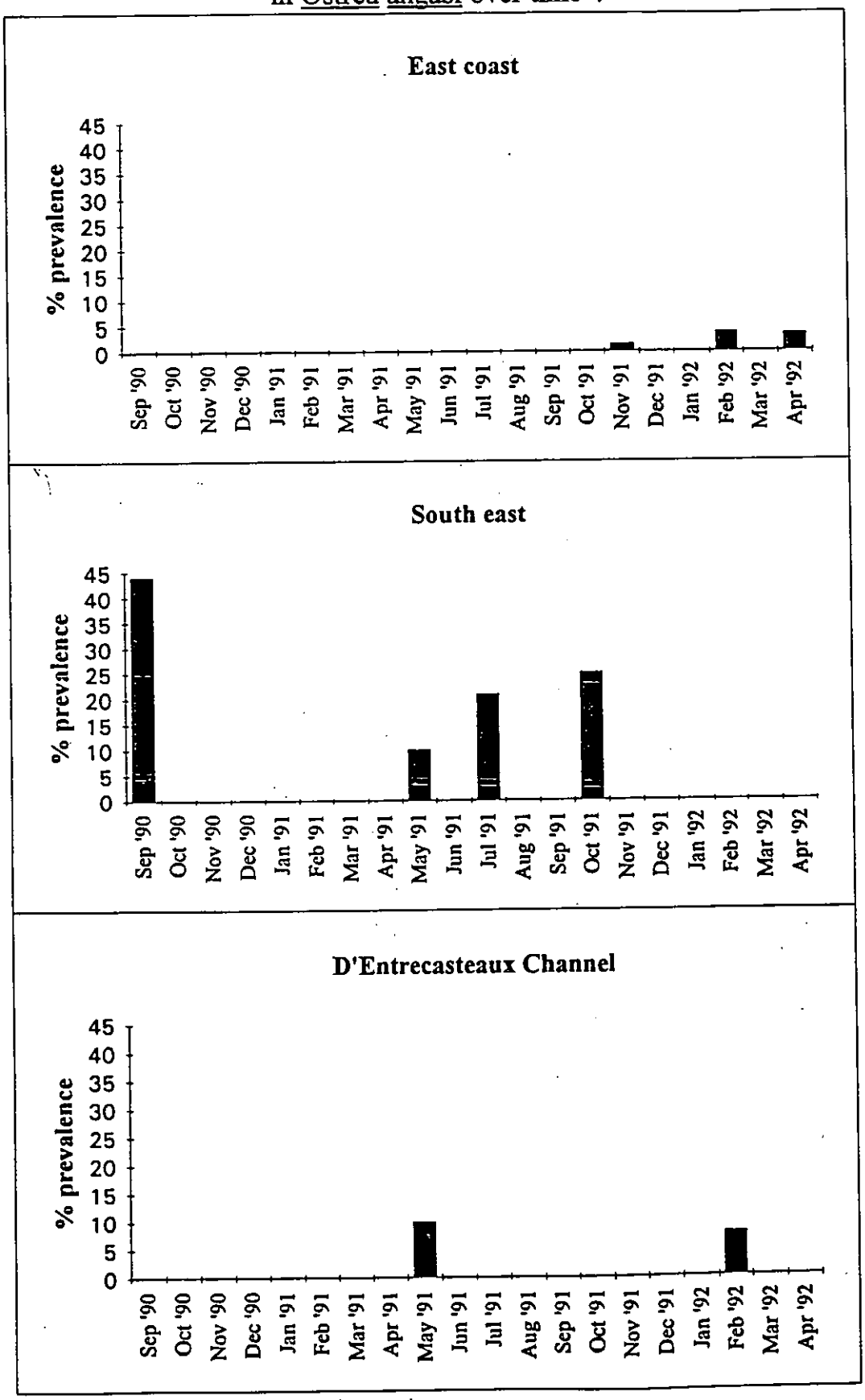


Fig 3.6: Variation in prevalence of Ancistrocoma sp in Ostrea angasi over time*.



*Not present in samples from the north west.

Data for the prevalence of *Trichodina* fitted the model age + area + season + age x season + area x age + season x age. Prevalence on the east coast was significantly lower than in any other area throughout the year.

Trichodina sp was not recorded from flat oyster gills.

Unidentified protozoans

Also present on the gills of <1% of Pacific oysters were two protozoans. Type A resembled the ciliate *Sphenophyra* sp and was present on the gills of 17 Pacific oysters. An unidentified organism (Type B) was also present on the gills of 8 Pacific oysters.

Flat oysters were not infected with any of these gill organisms.

Metazoa

Turbellarians

Turbellarians were found in the digestive system of 4 Pacific oysters from areas 1, 2 and 3. These organisms were only found during the months of January, February, March and April. Again, flat oysters were not infected.

Polychaetes

Shellblisters were apparent as raised blisters on the inside of the shell formed by the deposition of shell material by the oyster in reponse to the presence of a polychaete worm. They were found in 4% of Pacific oysters and 20% of flat oysters and were recorded from all growing areas. Figures 3.7 and 3.8 show the prevalence of shellblisters over the study period in each of the four growing areas for *C. gigas* and *O. angasi* respectively.

Data for the prevalence of shellblisters in Pacific oysters fitted the most complex model (Table 3.3) of age of host, season, and area and all second and third order interactions. Specifically, on the east coast, Pacific oysters of the "mature" age group had a significantly higher number of shellblisters than the younger age group in all seasons except spring. During summer, prevalence of shellblisters in the older east coast group was significantly higher than in any other area and during autumn the level was significantly higher than the northwest and the southeast

regions. Young oysters in the south east were significantly more heavily infected during autumn and winter than they were in summer. The level of infection in younger south east oysters was significantly higher than that of a comparable age group on the east coast but not compared to the D'Entrecasteaux Channel or the northwest.

The model for shellblisters in flat oysters indicated that prevalence was influenced by age of host, area and season and the interaction of age x season and area x season. However, there were no significant differences in stepwise comparisons of these categories. Again, this is probably due to the relatively low sample sizes contained in each category. From Fig 3.8 it appears that highest prevalence was in oysters from the east coast although consistent seasonal trends were not observed.

The polychaete fauna associated with shellblisters in Pacific and flat oysters is shown in Table 3.6. Species and prevalence of polychaetes found within the mantle cavity or on the surface of oysters are listed in Table 3.7.

During the period August 1990 - June 1991, 97 polychaetes representing 5 families were dissected from shellblisters. Members of one family - Family Spionidae - comprised 92.8% of all specimens dissected from shellblisters during this period. Three species of spionid polychaete were found, *Polydora websteri*, *P. hoplura* and *Boccardia chilensis*. Of these, *P. websteri* was the most common species with a prevalence of 55.7% (Table 3.6). Four specimens of the Family Neredidae and one each of the Families Ophelidae, Terebellidae and Polynodidae were also recovered from shellblisters.

A diverse polychaete fauna was recovered from the shell surface or mantle fluid of oysters. Nine families of polychaete were represented with the Family Nereididae comprising the majority (57%) of the fauna (Table 3.7).

Fig 3.7: Variation in prevalence of shellblisters in Crassostrea gigas over time.

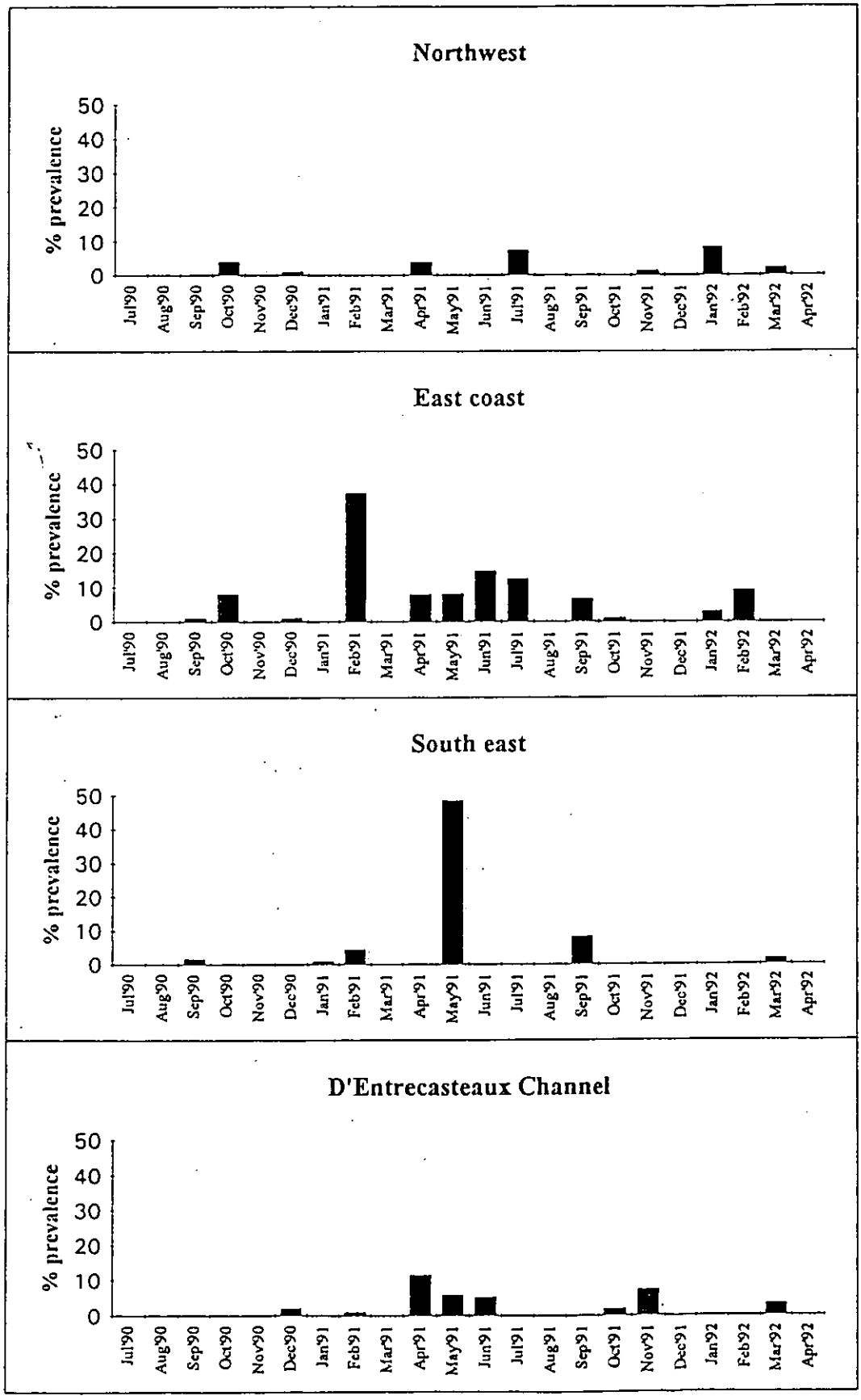


Fig 3.8: Variation in prevalence of shellblisters in Ostrea angasi over time.

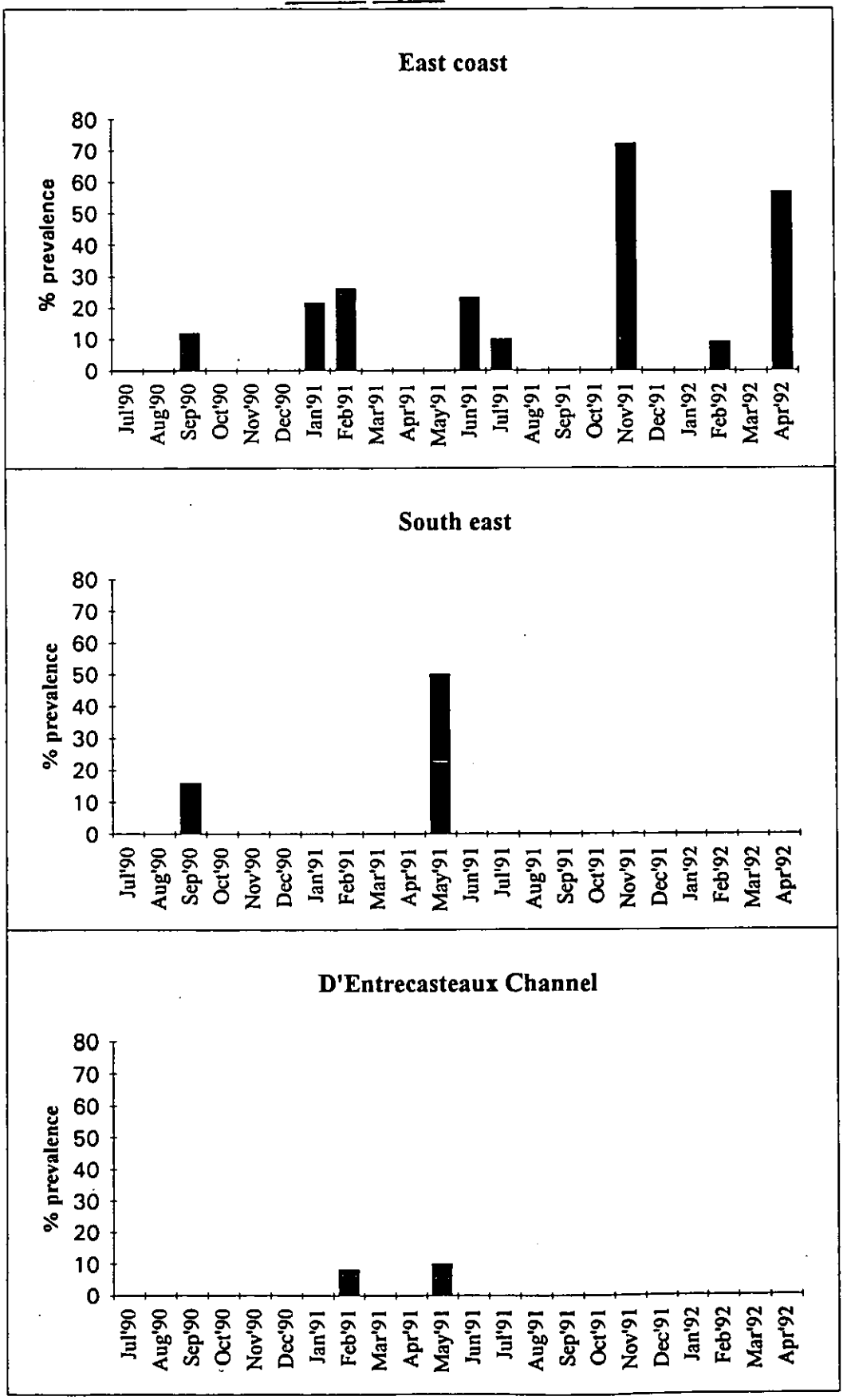


TABLE 3.6: Polychaetes recovered from shellblisters for the period August 1990 - June 1991

Species	Number from Pacifics and (flats)	Percentage (%)
Family Spionidae	**[90]**	**[92.8]**
Polydora websteri	51 (3)	55.7
Polydora hoplura	17	17.5
Boccardia chilensis	8	8.2
unspeciated	10 (1)	11.3
Family Nereididae	**[4]**	**[4.1]**
Nereis sp	1	1.0
Perenereis sp	2	2.1
unspeciated	1	1.0
Family Ophelidae	**[1]**	**[1.0]**
Armandia intermedia	1	1.0
Family Terebellidae	**1**	**1.0**
Family Polynoidae	**1**	**1.0**
TOTAL	**[97]**	**[100]**

TABLE 3.7: Polychaete fauna on the outside of the shell or in the mantle fluid for the period August 1990 - June 1991.

Species	Total Number	Percentage %	Number on shell	Number in mantle fluid
Family Spionidae	[15]	[16.1]		
Polydora websteri	2	2.2	2	
Polydora hoplura	3	3.2	2	1
Boccardia chilensis	6	6.4	3	3
unspeciated	4	4.3		4
Family Nereididae	[53]	[57.0]		
Neanthes vaalii	32	34.4	22	10
Neanthes sp	5	5.4	4	1
Pseudonereis sp.	5	5.4	5	
Platynereis sp 1	2	2.2	2	
Namanereis sp	1	1.1		1
SubFamilyGymnonereidinae				
Nicon sp?	1	1.1		1
unspeciated	7	7.5	1	6
Family Terebellidae	[8]	[8.6]		
Nicolea amnis	3	3.2	3	
Streblosoma sp	4	4.3	4	
unspeciated	1	1.1		1
Family Serpulidae	[3]	[3.2]		
Galeolaria caespitosa	3	3.2	3	
Family Cirratulidae	[9]	[9.7]		
Cirriformia filigera	2	2.2	2	
Cirriformia capensis	7	7.5	7	
Family Syllidae	[2]	[2.2]		
Typosyllis armillari	2	2.2	2	
Family Polynoidae	[1]	[1.1]		
Harmothoe praeclara	1	1.1	1	
Family Chrysopetalidae	[1]	[1.1]		
Paleonotus sp	1	1.1	1	
Family Eunicidae	[1]	[1.1]		
Eunice sp	1	1.1	1	
TOTAL	[93]	[100]	**75**	**18**

Crustaceans

Pseudomyicola spinosus

Crustaceans resembling *Pseudomyicola spinosus* were found in 3.2% of Pacific oysters (Fig 3.9) and 3.7% of flat oysters (Fig 3.10) from all areas.

The model which best fitted the data for Pacific oysters showed that incidence of *Pseudomyicola spinosus* was determined by age, area and season and the interaction of area x season. Stepwise comparisons showed infections in Pacific oysters were significantly higher on the east coast and northwest coast than in other areas during summer. During autumn and spring the level on the east coast was significantly higher than the channel and the southeast respectively. At other times of the year there was no significant difference between areas or seasons.

In flat oysters, prevalence of *P. spinosus* was determined by age and area, and their interaction. However, there were no significant differences in stepwise comparisons between categories. From Fig 3.10, numbers were higher on the east coast.

Gut copepods

Of the 1500 Pacific oyster guts dissected, 8 were infected with copepods and in two oysters, these copepods were also present in the digestive gland. The copepods were not identified, but were noted to be red in colour. These copepods may be the same type (*Pseudomyicola spinosus*) seen in these tissues in histological sections. Infected oysters were collected from the D'Entrecasteaux Channel in autumn of 1991.

No parasites or other organisms were found in the gut of the 183 flat oysters during dissection although, as noted above, organisms were sometimes found during histological examination.

Fig 3.9 : Variation in prevalence of Pseudomyicola sp in Crassostrea gigas over time.

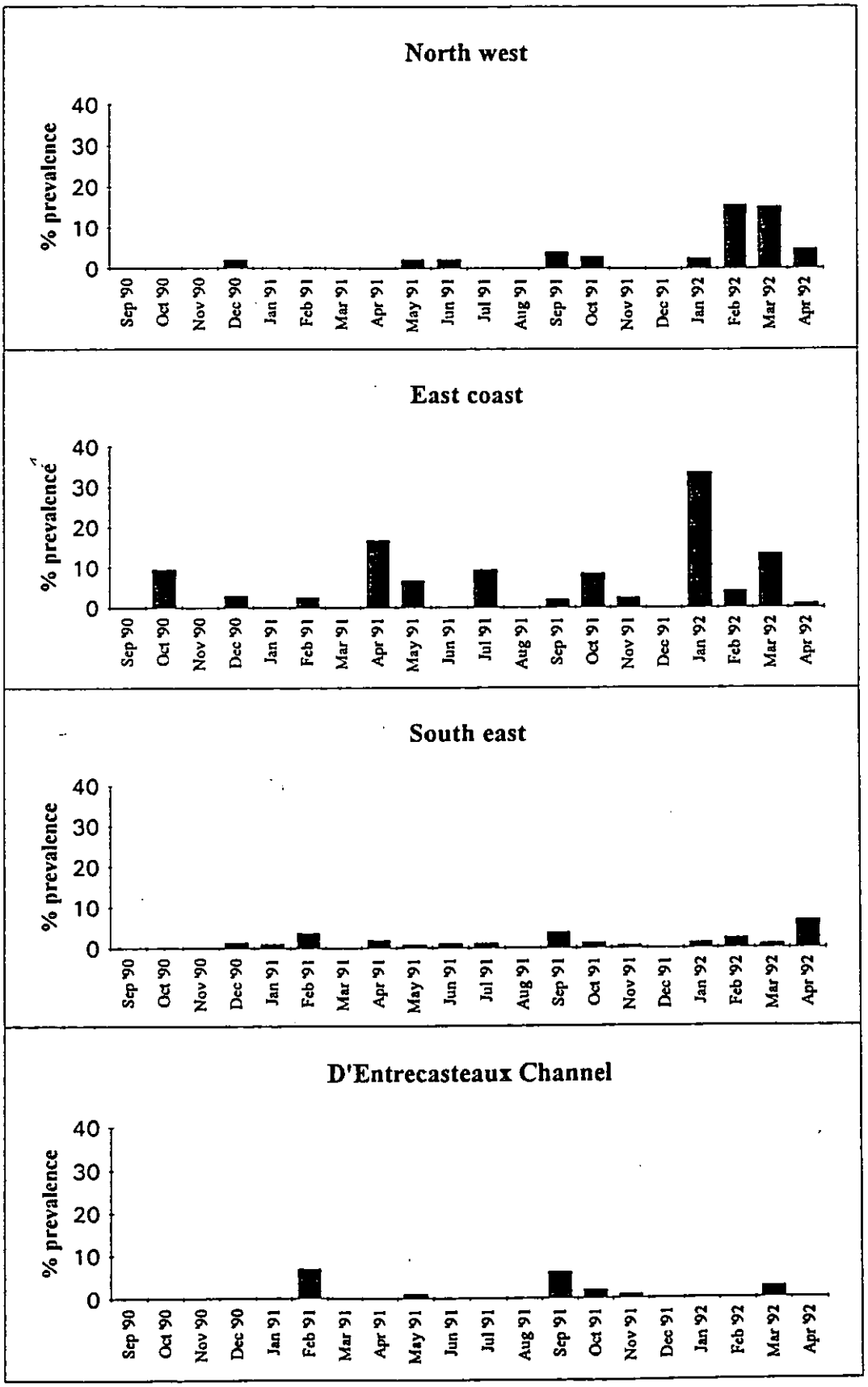
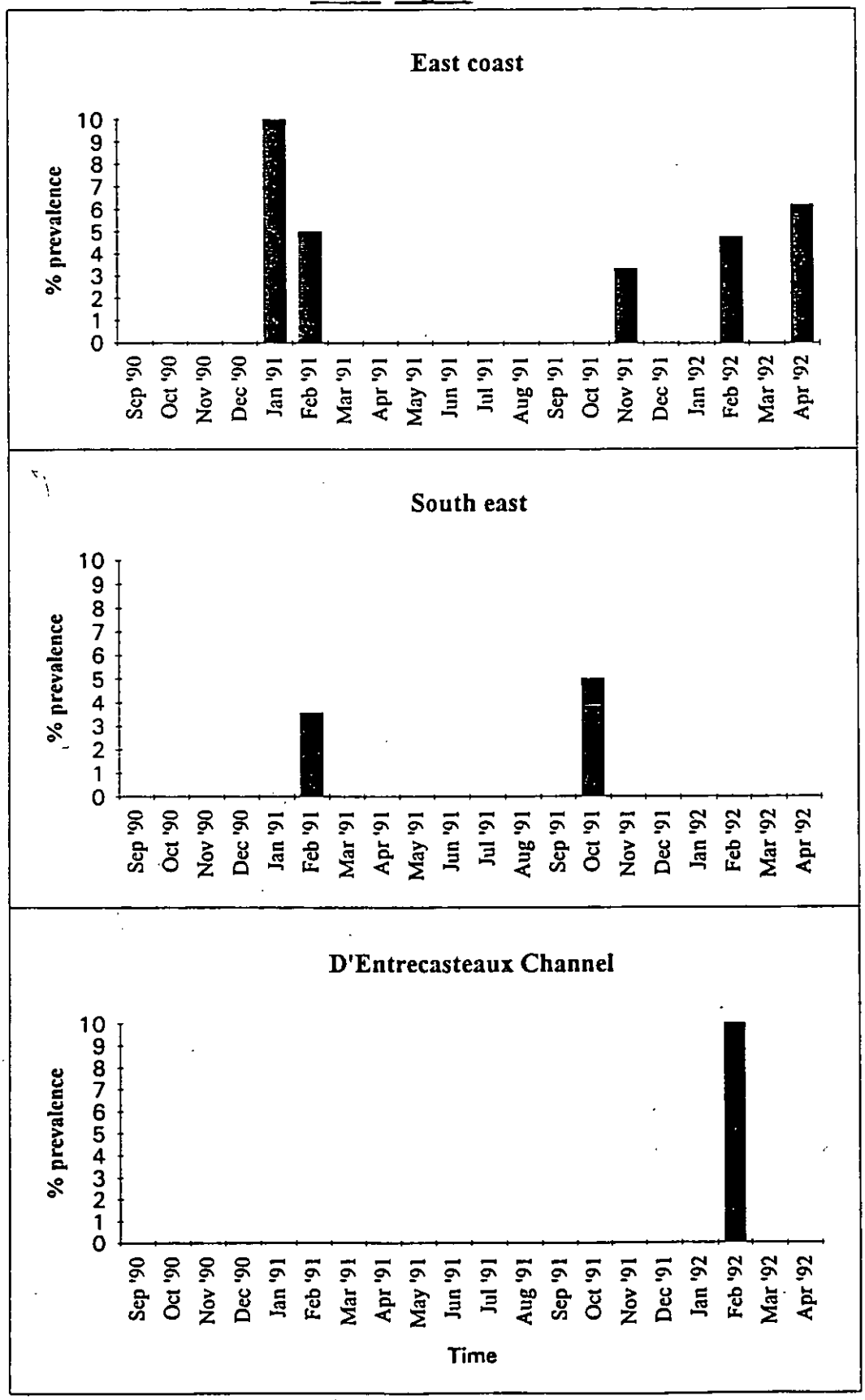


Fig 3.10: Variation in prevalence of Pseudomyicola sp in Ostrea angasi over time*.



*Not present in samples from the north west

Gill copepods

Copepods were found invading the gills of 0.1% Pacific oysters and were only detected in samples from the northwest and eastcoast. One flat oyster from the southeast was similarly infected.

Abnormal Conditions

Watery condition

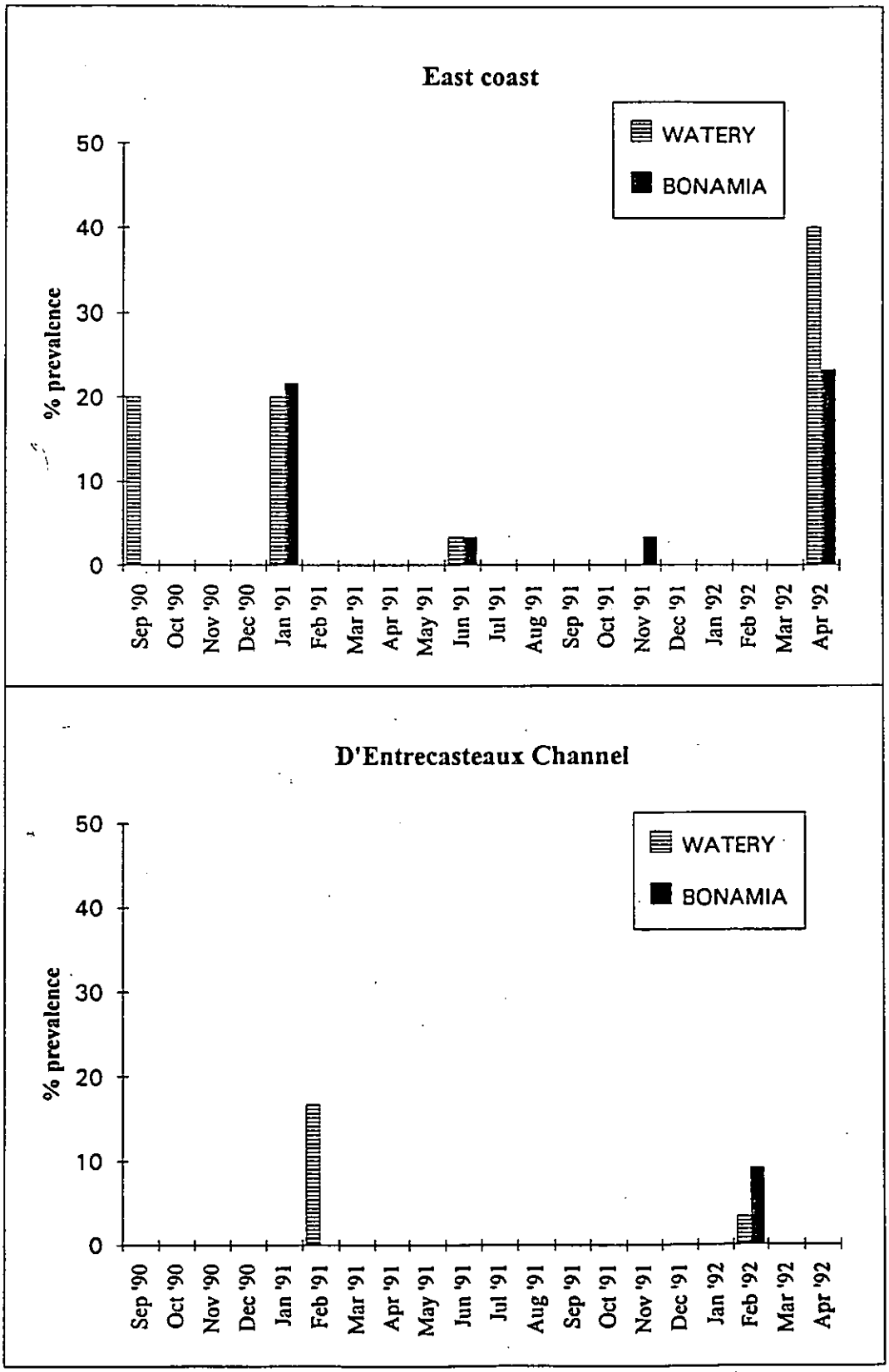
One hundred and thirty five Pacific oysters (2.5%) and 88 flat oysters (14%) appeared watery or emaciated upon opening. This condition was observed in samples from all areas from both species of oyster. From the GLM the incidence of watery specimens for Pacific oysters was determined by age, area and season and the interaction of area x season. Numbers of watery specimens were significantly higher in the D'Entrecasteaux channel than the east coast and northwest during spring and summer. Watery oysters were more prevalent in summer than spring for the D'Entrecasteaux Channel. Conversely, higher numbers of watery oysters were found in spring than summer for the southeast region.

The number of watery flat oysters was influenced by age and season and the interaction of age x season. Significantly higher numbers of watery flat oysters were seen in autumn than spring and summer for the 2-3 year age group. Flat oysters with a watery appearance were often noted in those samples which were infected with *Bonamia* sp (Fig 3.11).

Mortality

Information regarding the levels of mortality for Pacific and flat oysters, requested from selected farmers keeping accurate records in each region is shown in Table 3.8. For the purposes of this study, this data was available from July 1990. For Pacific oysters the levels ranged from 0 - 11% per year with an average mortality over all areas of 5.3%. Similarly for flat oysters, the levels ranged from negligible to 20% per year with an average for all areas of 6.25%. One lease in the D'Entrecasteaux Channel reported relatively high mortality (15-20%) in stocks of *O. angasi* during July 1990 - May 1991. Although at this time the farm was receiving *Bonamia* sp infected stock, the mortality had been attributed to loss of gear and starfish predation.

Fig 3.11: Variation in prevalence of a watery condition or Bonamia sp in Ostrea angasi over time*.



* Neither condition found in samples from the north west or the south east.

Additionally, farmers with leases in Georges Bay infected with *Bonamia* sp were asked to give mortality figures for their stocks of *O. angasi*. These figures were 10-15% and 20-40% for oysters held subtidally and intertidally, respectively. It should be stressed that these figures were based on estimates given by the farmers and may be inaccurate.

Table 3.8: Mortality data collected from selected farms during the survey.

SPECIES	DATE	AREA 1	AREA 2	AREA 3	AREA 4
Pacifics	July-Sept 1990	up to 5%	up to 10%	4%	2-10%
	Oct-Jan 1991	0-5% (av 1.2%)	5%	6.7%	5%
	Feb-May 1991	0-6% (av 1.5%)	6.5%	0-8.5% (av 5.4%)	5%
	Jun-July 1991	n/a	n/a	n/a	n/a
	Sep-Nov 1991	0-6.5% (av 1.5)	5%	4.5%	7%
	Dec-Feb 1992	4.4%	4% (7%*)	3.2%	9%
	Mar-June 1992	<1%	4% (7*)	3%	11%
Flats	July-Sept 1990	n/a	8-10%	neg	20%
	Oct-Jan 1991	n/a	5%	neg	15%
	Feb-May 1991	n/a	5%	neg	15%
	June-July 1991	n/a	n/a	n/a	n/a
	Sept-Nov 1991	n/a	10%	<1%	<1%
	Dec-Feb 1992	n/a	1%	2%	n/a
	Mar-June 1992	n/a	1%	<2%	n/a

3.2 QUALITATIVE RESULTS

Ovacystis

Ovacystis infections were found in Pacific oysters in both male and female gametes primarily in oysters in the maturing stages of gametogenesis.

In females, infections were characterised by hypertrophied, opaque, finely granular, eosinophilic nuclei with marginated chromatin (Fig 3.12 and 3.13). These infected nuclei measured, on average, between 27.9 µm (95% C.I. = 3.3 µm) x 35.0 µm (95% C.I. = 4.5µm). In most cases, only a few ova were affected in an individual oyster, although in partially spent females sometimes a large proportion of remaining ova were infected. The cytoplasm of infected cells was scanty and eosinophilic.

In males, the infection was similar in morphology (Fig 3.14) with hypertrophied, finely granular nuclei with marginated chromatin and eosinophilic inclusions seen. Inclusions were roughly ovoid and were variable in size and measured on average 41.9 µm (95% C.I. = 5.1 µm) x 52.8 µm (95% C.I. = 4.2 µm) (Fig 3.15). It was not possible to determine whether infected cells were sperm cells or remnant ova although unaffected remnant ova were never observed in infected male oysters.

Deparrafinised material examined by electron microscopy showed the presence of viral particles measuring between 38.5 and 48.1 nm in diameter (Figs 3.16 and 3.17). These particles were consistent with the morphology of papovavirus particles in that they were of a consistent size 38 - 43 nm diameter, were icosahedral, and were apparently non-enveloped. In one case viral particles were observed in a membrane bound vesicle which had presumably budded from the nucleus.

In addition to these very large inclusions, the ova of some oysters (3.5%) were noted with more darkly staining nuclei. In these cells, the cytoplasm was more basophilic, but had fewer granules giving an overall paler appearance. Oysters with this appearance were categorised as having "abnormal ova". Farley (1985) noted that similar cells in *Crassostrea virginica* and suggested that these were an early stage of the papovavirus infection. Figure 3.18 shows a follicle containing one such ovum and an ovum with a more advanced stage of ovacystis.

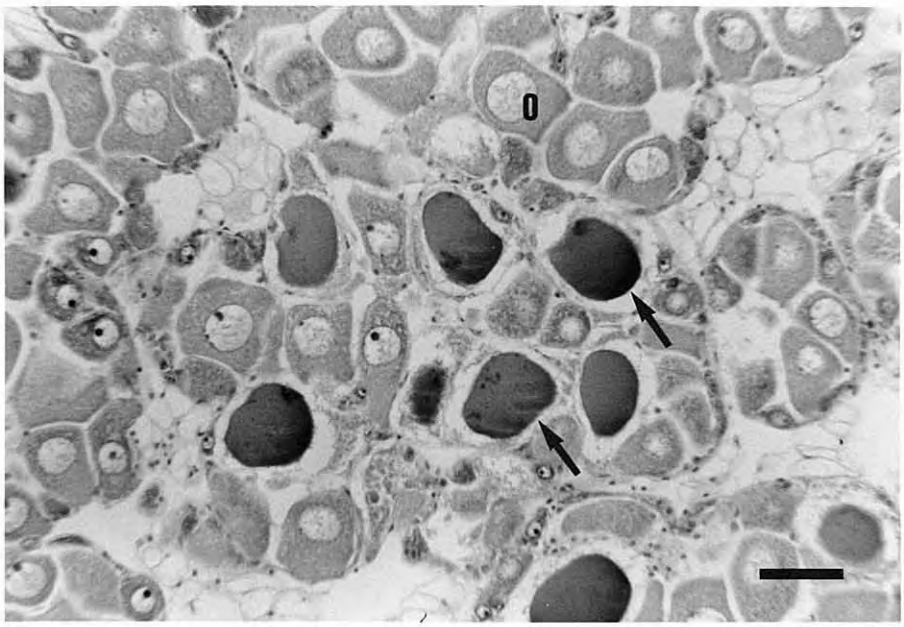


Fig 3.12: Maturing ova (O) of *Crassostrea gigas* with ovacystis (papovavirus) inclusions (arrows) in the nuclei (H & E). Scale bar = 50μm.

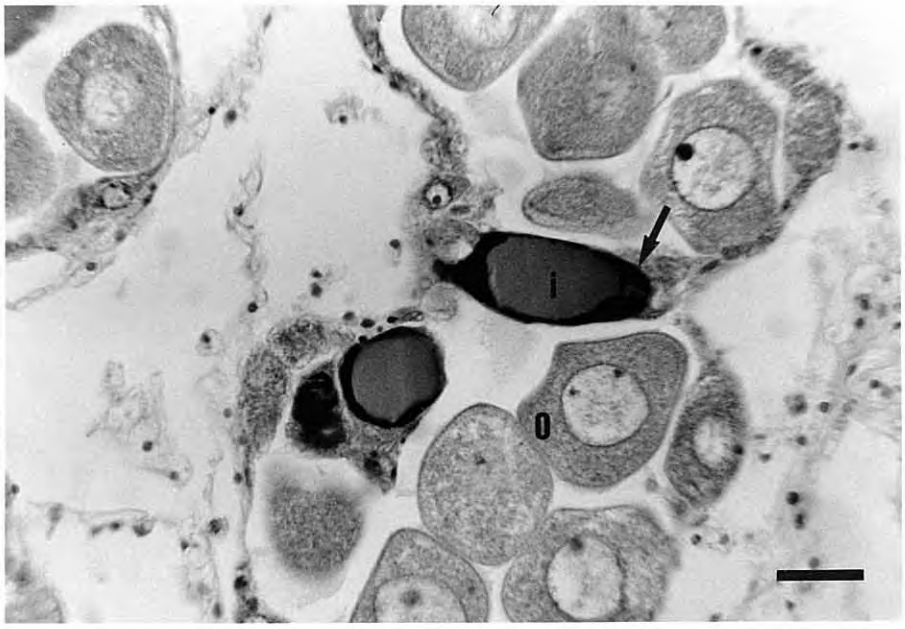


Fig 3.13: Ovacystis inclusions in the ova of *Crassostrea gigas*. Note the marginated chromatin (arrow) and the finely granular appearance of the inclusion (i) compared to normal ova (O) (H & E). Scale bar = 25μm

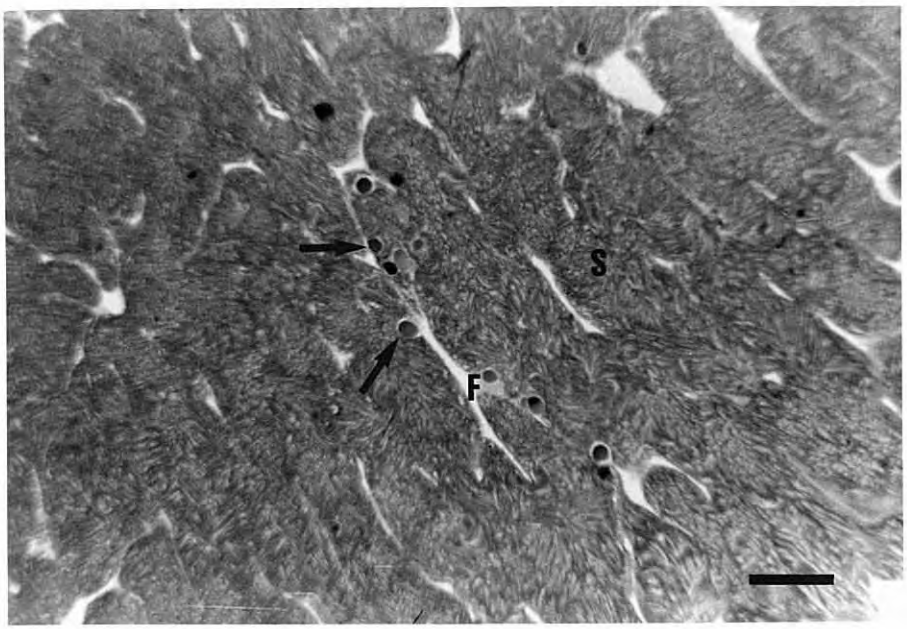


Fig 3.14: **Fully mature male *Crassostrea gigas* with ovacystis inclusions (i) located at the edge of the gonad follicle (F); S = sperm (H & E). Scale bar = 250μm.**

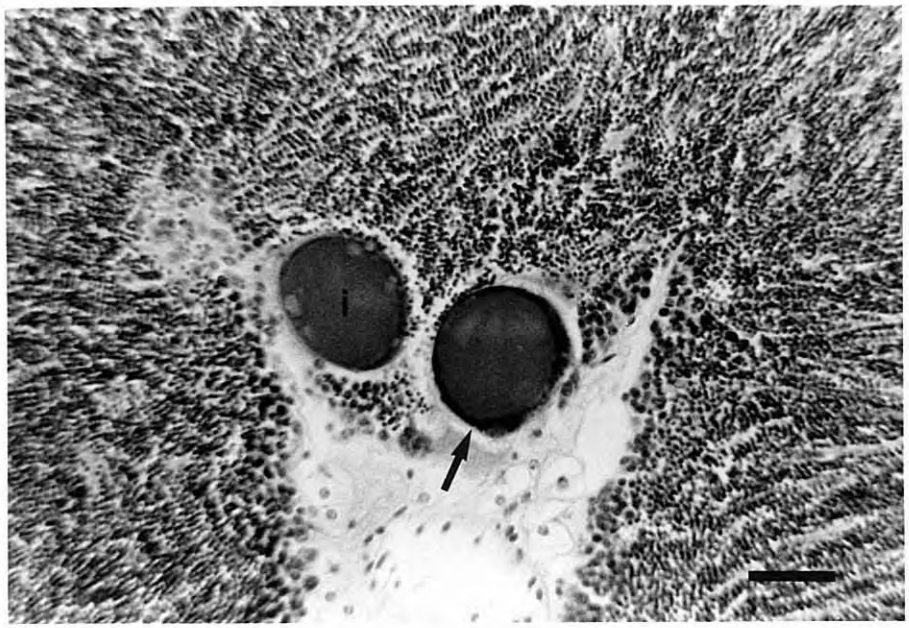


Fig 3.15: **High magnification micrograph of ovacystis inclusions in male *Crassostrea gigas*. Inclusions (i) have marginated chromatin (arrow) and finely granular appearance (H & E). Scale bar = 25μm.**

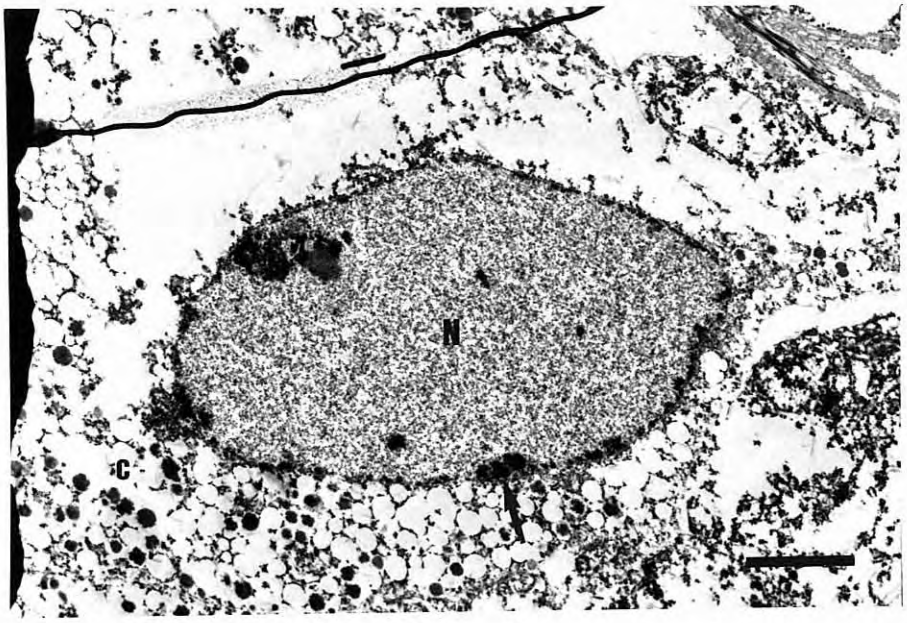


Fig 3.16: Low power electron micrograph of papovavirus infected ovum of *Crassostrea gigas*. Note the marginated chromatin (arrow) at the edge of the nucleus (N). Scale bar = 5 μm.

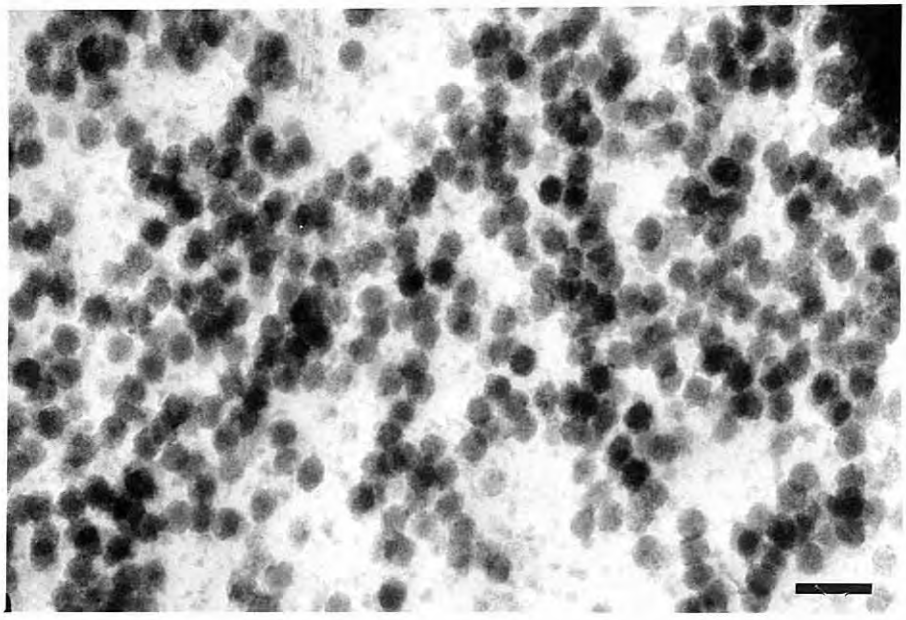


Fig 3.17: High power electron micrograph of papovavirus particles from the nucleus of an infected ovum. Scale bar = 150 nm.

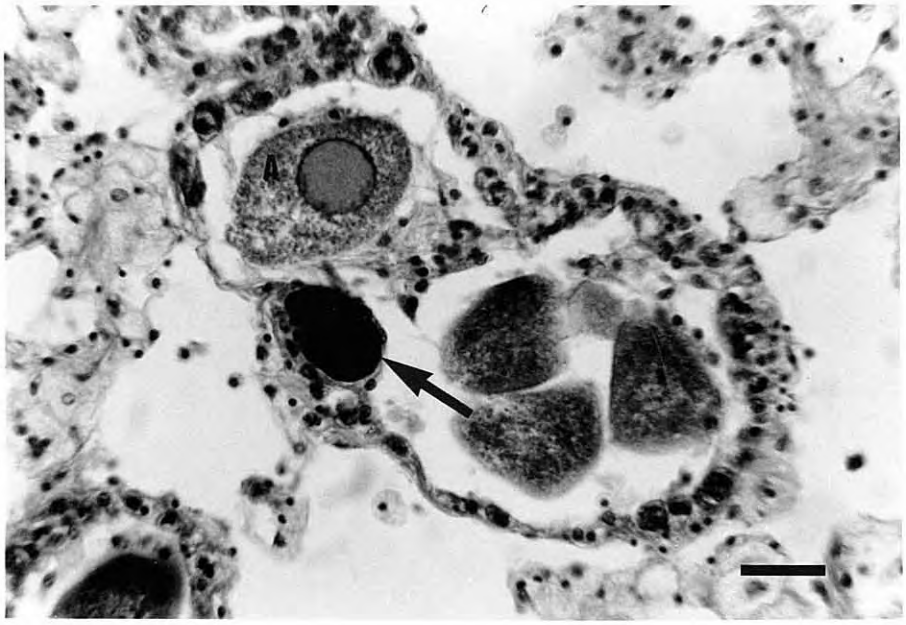


Fig 3.18: Partially spent ova of *Crassotrea gigas* with follicle containing one well developed ovacystis inclusion (arrow) and an "abnormal" ovum (A) with less dense cytoplasm, and an opaque, marginated nucleus (H & E). Scale bar = 25μm.

Haemocytic response to the presence of either of these types of inclusions was not seen. Due to the low prevalence, intensity and absence of host response to the inclusions, ovacystis infections are not considered to be detrimental to Pacific oyster health.

Virus X

Intranuclear inclusions present in the connective tissue cells of one flat oyster, were located near the gonad tissue and nearby regions of the digestive gland. Electron microscopy of identical inclusions in wild *O. angasi* showed herpes-like viral particles (Handlinger pers comm).

Inclusions were abundant in the tissue with up to 5 inclusions were seen in a high power (100x obj.) field of view.

The inclusions were associated with an intense haemocytic response especially surrounding the gonad tissues and digestive gland (Fig 3.19). Also, digestive epithelial height was reduced in this infected oyster. Affected cell nuclei had marginated chromatin and eosinophilic inclusion bodies which were surrounded by a halo (Fig 3.20).

The intensity of infection and pathology associated with the inclusions suggest that this infection is detrimental to its host. However, due to its extemely low prevalence in flat oyster stocks and in the absence of reported mortality in the stock from which this infected oyster was collected, it is thought that this virus does not pose an immediate risk to the culture of flat oysters in Tasmania.

Rickettsial inclusions

Large, basophilic finely granular inclusions were seen in the digestive gland epithelium of Pacific and flat oysters, and in the gut epithelium and connective tissue of Pacific oysters.

Pacific oyster inclusions

Histologically, there appeared to be four morphological types of inclusions in the digestive gland epithelia of Pacific oysters. The first three types were found

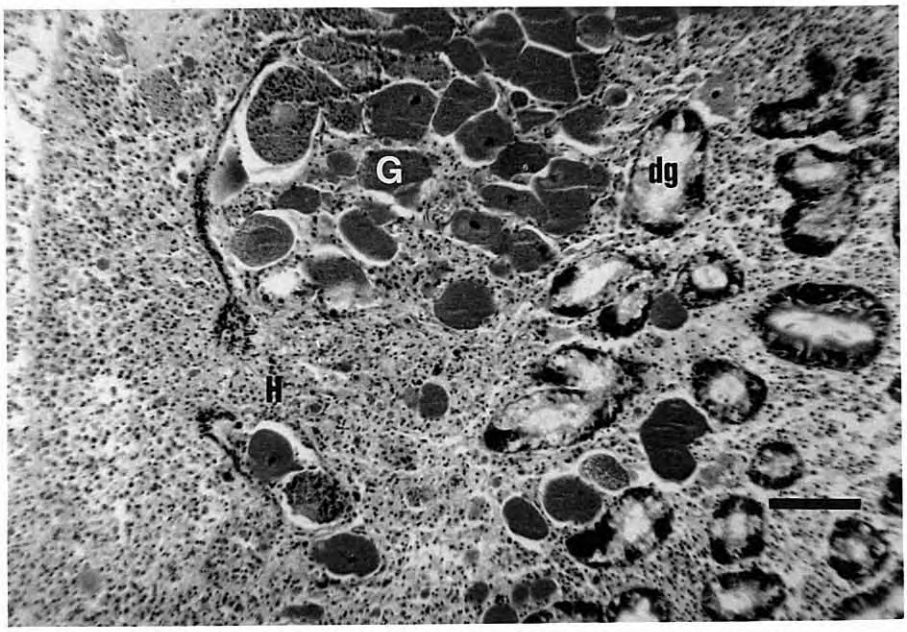


Fig 3.19: Section through gonad (G) and digestive gland (dg) of *Ostrea angasi* with intranuclear inclusions. Note the intense diffuse haemocytic infiltration (H). Scale bar = 100 μm.

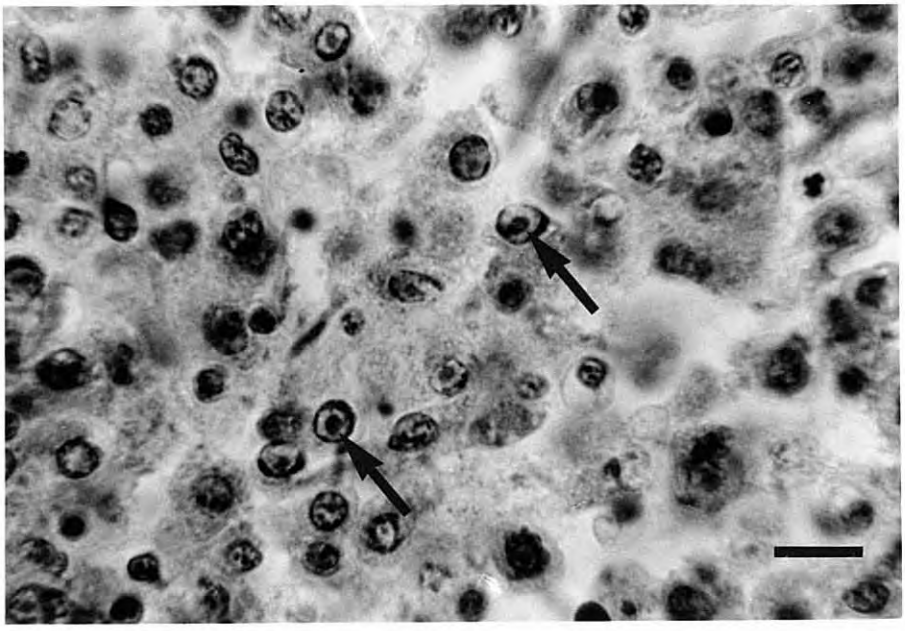


Fig 3.20: High power micrograph of intranuclear inclusions (arrows) in cells infiltrating the connective tissue. Scale bar = 10 μm.

in samples collected throughout Tasmania. A single specimen infected with the fourth type was found from the south east on the 19.11.91.

Type A_1 were granular, basophilic intracytoplasmic inclusions of variable size with the largest inclusions measuring 11.3 µm (95% C.I. = 2.7 µm) x 9.3 µm (95% C.I. = 2.7 µm). They were located throughout the height of the absorptive cells of the non-ciliated digestive tubule epithelia (Fig 3.21) and were sometimes present within a vacuole (Fig 3.22). In these cases the inclusions were surrounded by a halo. These inclusions were found in 0.5% of all Pacific oysters examined i.e. 35% of all Pacific oyster inclusions were of this type. Infections were regionalised with up to 23 inclusions seen in a low power (10x obj.) field of view (3.63mm^2).

Only this type of inclusion from Pacific oysters was examined using transmission electron microscopy. The inclusions were comprised of prokaryotic, rod shaped organisms (Fig 3.23). The organisms were double membrane bound and measured between 380 nm and 460nm in diameter (average 420 nm) and were between 620 nm and 1140 nm in length (average 850 nm) (Fig 3.23). In some inclusions, membrane outpocketings or "blebs" were seen adjacent to the rickettsial organisms (Fig 3.24). In addition, some organisms contained vacuoles which were not membrane bound (Fig 3.25) and some organisms in the process of division were observed (Fig 3.26). The morphological structure of these organisms seen by light and electron microscopy was similar to that previously described in the literature for rickettsiales-like organisms from shellfish (Comps et al., 1977; Elston and Peacock, 1984 and Buchanan, 1978).

The second type of inclusion A_2, was morphologically similar to type A_1 when viewed by light microscopy in that these were also granular, basophilic, intracytoplasmic inclusions in the epithelium of non-ciliated digestive tubules. However, these inclusions were uniform in shape, well defined and circular and measured 10.0 µm (95% C.I. = 1.1 µm) in diameter and were always located at the apex of the absorptive cells (Fig 3.27). They appeared to be contained within a vacuole although the halo around the inclusion was not as obvious as with type A_1 inclusions. Rarely were more than 3 of these inclusions found in a low power field i.e. 10x objective or more than one inclusion per digestive tubule observed. In one case, inclusions were seen in the lumen of the digestive tubule where they had presumably been expelled from the cell or had been present in a cell

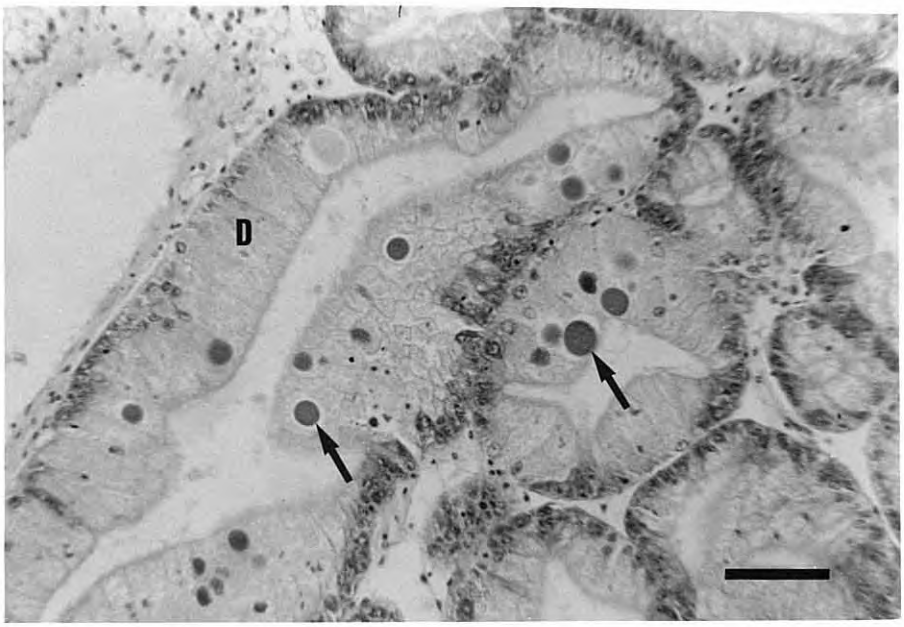


Fig 3.21: Digestive gland epithelia (D) of *Crassostrea gigas* containing Type A_1 rickettsial inclusions (arrows) (H & E). Scale bar = 50μm.

Fig 3.22: Type A_1 rickettsial inclusions (large arrow) within a vacuole (small arrow) of digestive gland epithelial cells of *Crassotrea gigas* (H & E). Scale bar = 20 μm.

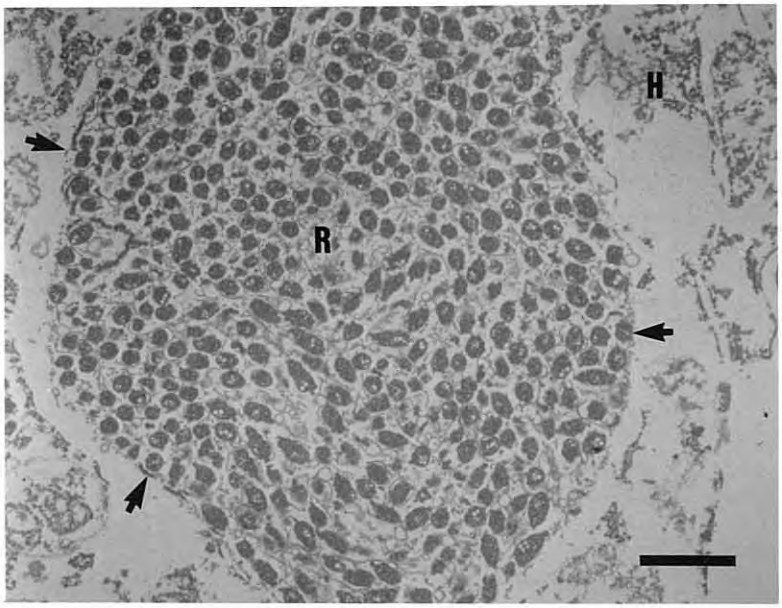


Fig 3.23: Electron micrograph of whole rickettsial inclusion (R) in digestive gland epithelia of *Crassostrea gigas*; H = host cell; margin of inclusion is indicated by arrows. Scale bar = 2 μm.

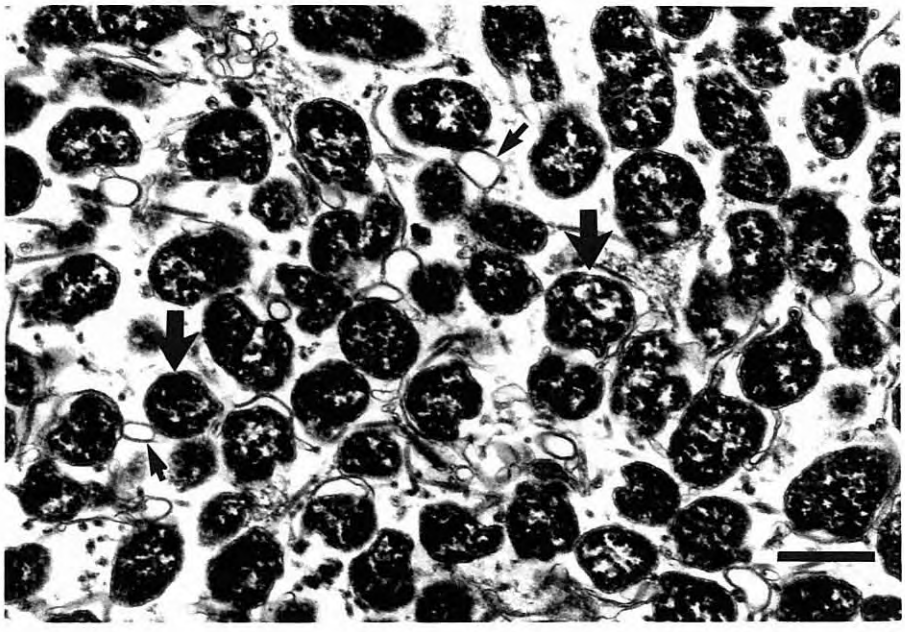


Fig 3.24: Rickettsial organisms (wide arrows) with membrane outpocketings ("blebs") (small arrows). Scale bar = 500 nm.

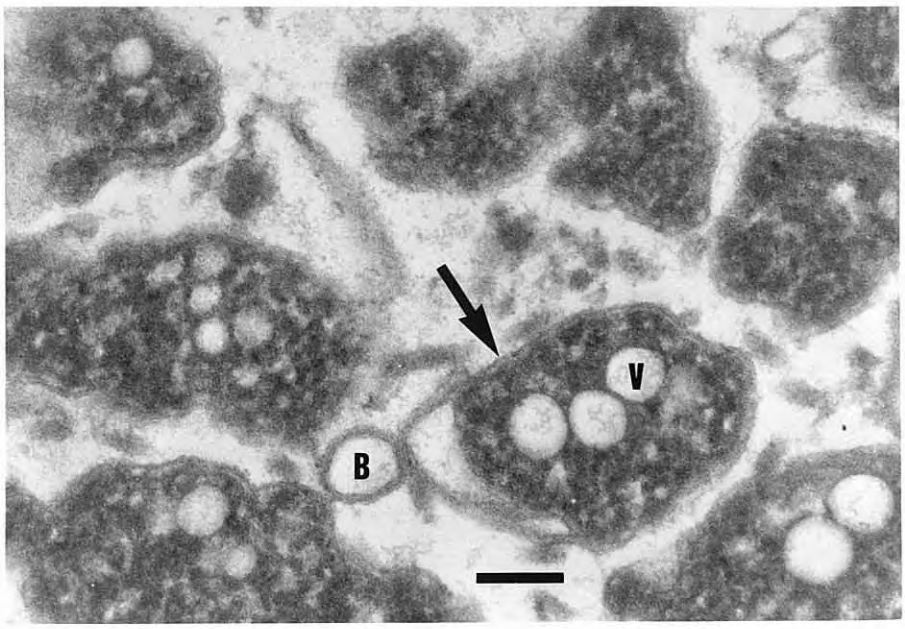


Fig 3.25: Electron micrograph of rickettsial organisms (arrow) showing non membrane bound vacuoles (V) and a membrane outpocketing or "bleb" (B). Scale bar = 150 nm.

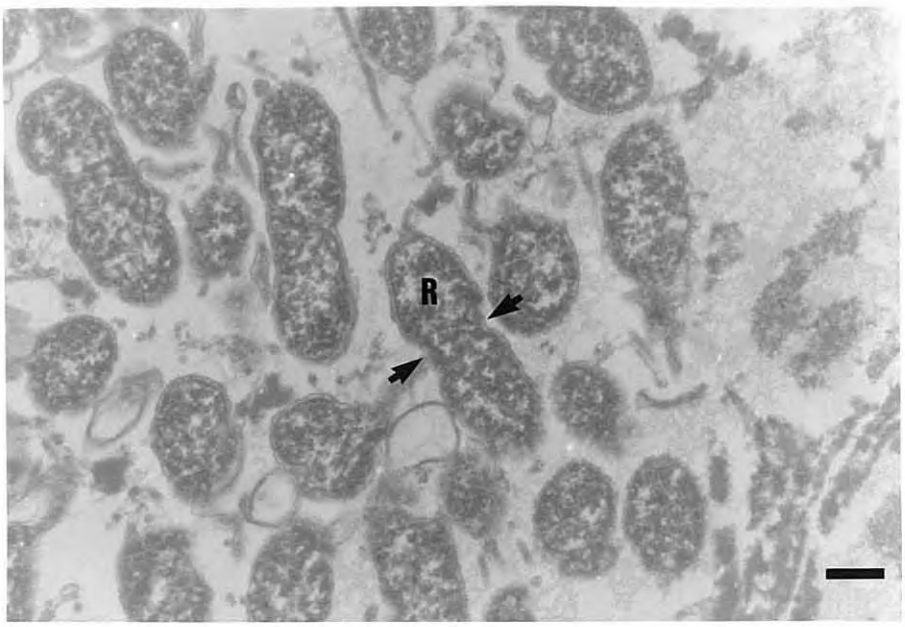


Fig 3.26: Electron micrograph of rickettsial organisms (R) in the early stages of binary fission (arrows). Scale bar = 250 nm.

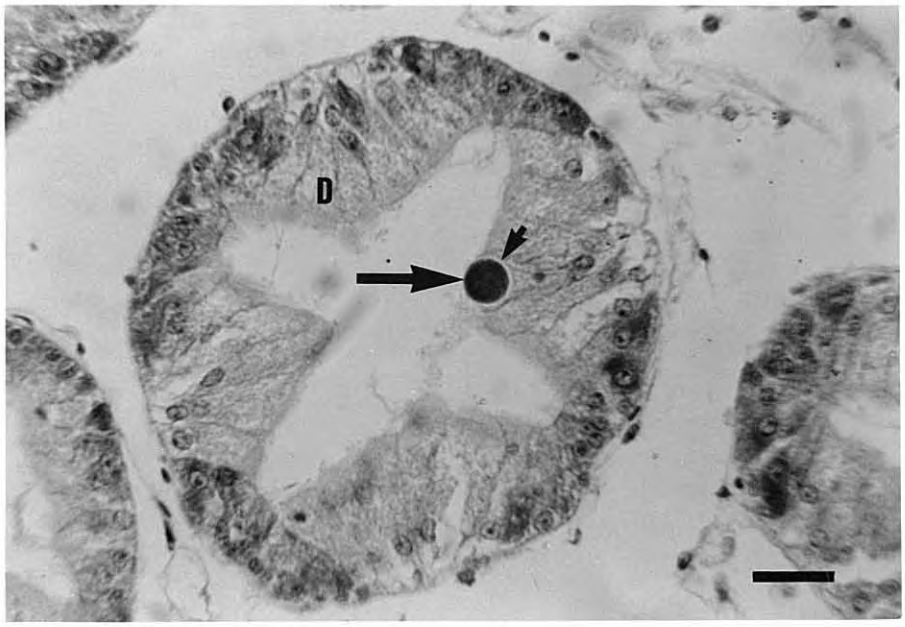


Fig 3.27: **Profile of a digestive tubule (D) of *Crassostrea gigas* containing Type A_2 rickettsial inclusion (large arrow). Note the vacuole (small arrow) surrounding the inclusion (H & E). Scale bar = 20 μm.**

which had sloughed from the tubule. These were the most common type of inclusions with 0.7% of Pacific oysters infected i.e. 50% of rickettsial inclusions in Pacific oysters were of this type.

The epithelia of ciliated digestive epithelium was infected with a third type of inclusion (Type B). These inclusions were darkly basophilic, oblong in shape and measured 12.8µm (95% C.I. = 1.6 µm) x 16.5 µm (95% C.I. = 2.3 µm) and were located close to the basal membrane (Fig 3.28). Some had a swirled appearance and were often located within a cytoplasmic vacuole and surrounded by a clear halo (Fig 3.29). Type B were less common than the previous two inclusion types with 0.2% of Pacific oysters infected i.e. 13.9% of Pacific oyster rickettsial inclusions were of this type. Between 0 and 3 inclusions were seen in a low power field with an average of 1.1 inclusions.

One oyster was found with inclusions similar to Type B inclusions, but these were found either in the connective tissue close to the digestive gland or immediately adjacent to the basal membrane of digestive tubules (Fig 3.30). These Type C inclusions measured 24.1 µm (95% C.I. = 4.9 µm) x 19.3 µm (95% C.I. = 3.5 µm) and had a swirled or fibrillar appearance (Fig 3.31). They sometimes appeared to be located in a vacuole although they were not as clearly defined as the Type B inclusions. Their location in the tissues was such that the presumptive host cell was not clearly defined. Up to 3 inclusions were seen in a low power field of view.

In addition, granular inclusions were occasionally found in tissue remote from the digestive tubule epithelium in Pacific oysters. Two Pacific oysters collected from the D'Entrecasteaux Channel on 20.11.91 had bean shaped, finely granular, slightly basophilic inclusions measuring 22.0 µm (95% C.I. = 4.7 µm) long and 9.2 µm (95% C.I. = 1.8 µm) wide in the epithelium lining the stomach (Type D) (Fig 3.32). Rarefication of the cytoplasm surrounding the inclusion was noted. Inclusions were located regionally within the stomach wall and where present, an average of 7 inclusions were seen in a low power field.

Roundish, granular inclusions were seen in the connective tissue of another two Pacific oysters (Type E). These inclusions varied in size from 18.9 µm to 107.1 µm in diameter with an average of 45.4 µm (95% C.I. = 25.1 µm) and had indistinct edges (Fig 3.33). The infected oysters were collected from the northwest on 16.3.92 and east coast on 13.5.91. A slight host response

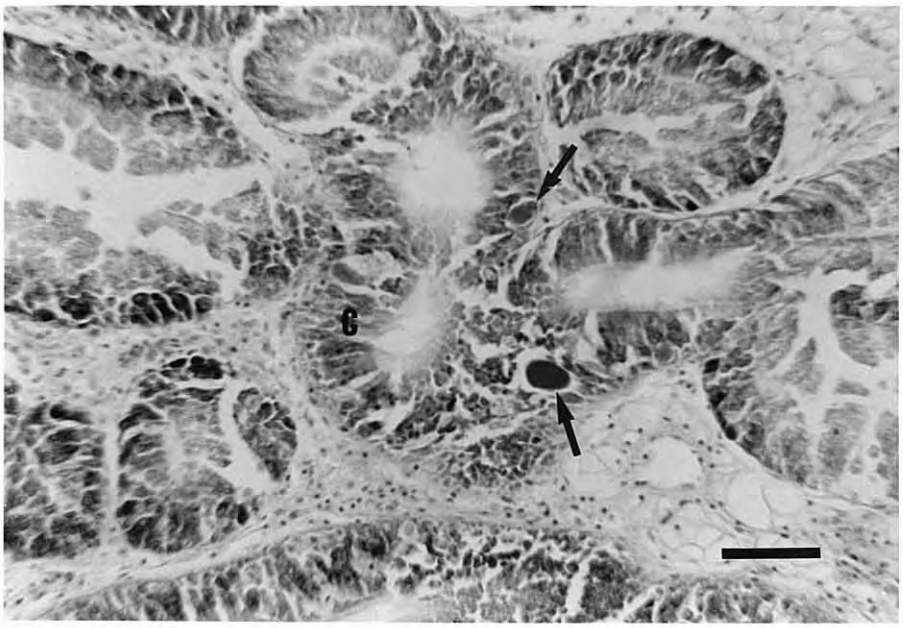


Fig 3.28: Type B rickettsial inclusions (arrows) in the ciliated digestive tubule epithelia (C) of *Crassostrea gigas* (H & E). Scale bar = 50 μm.

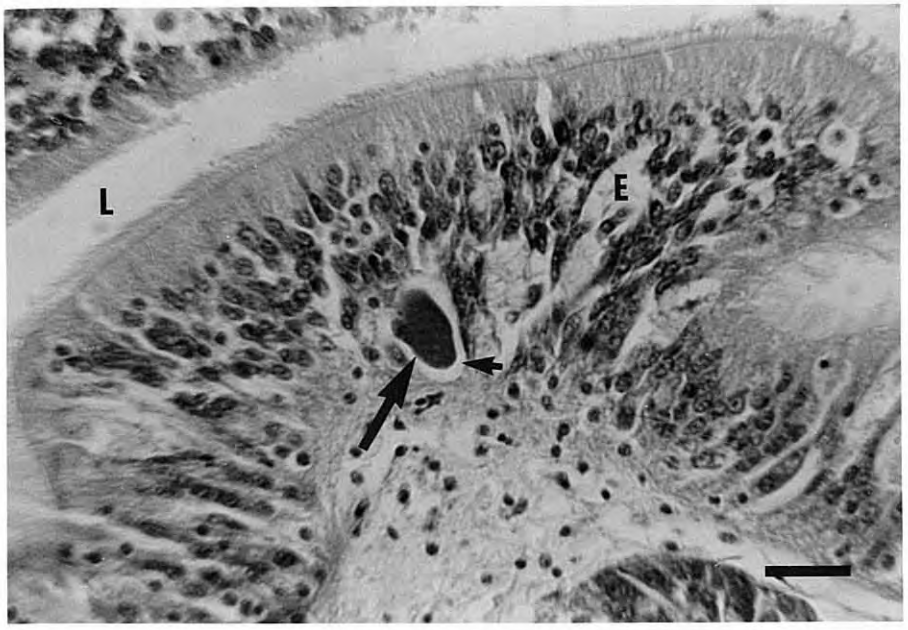


Fig 3.29: High power micrograph of ciliated digestive epithelium (E) containing Type B rickettsial inclusion (large arrow) located within a vacuole (small arrow); L = lumen of digestive tubule (H & E). Scale bar = 20 μm.

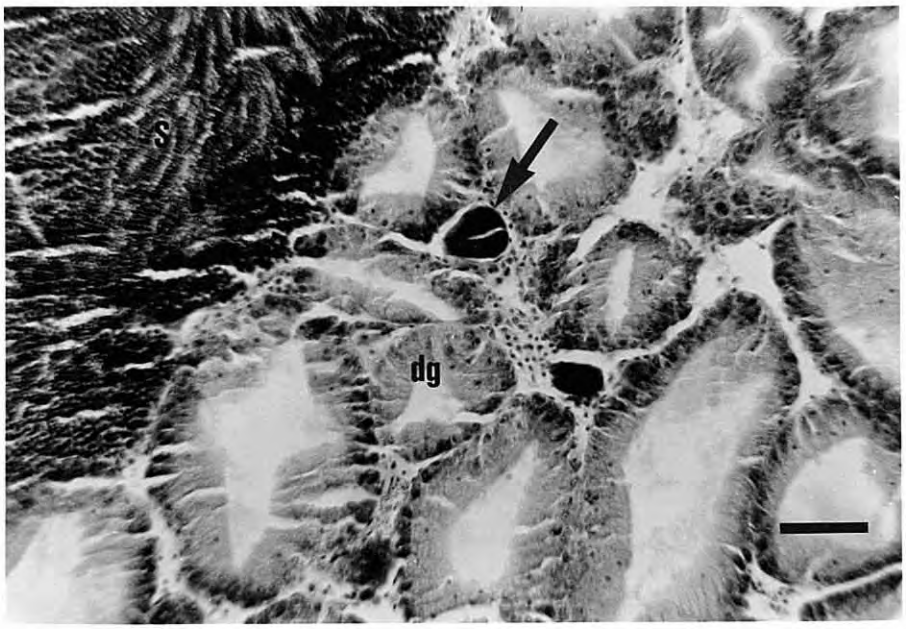


Fig 3.30: Profile of digestive gland (dg) of *Crassostrea gigas* showing Type C rickettsial inclusions (arrow) (H & E). Scale bar = 50 μm.

Fig 3.31: High power micrograph of Type C rickettsial inclusion near the ciliated digestive epithelium (E) of *Crassostrea gigas*; H = infiltration of haemocytes. Scale bar = 25 μm

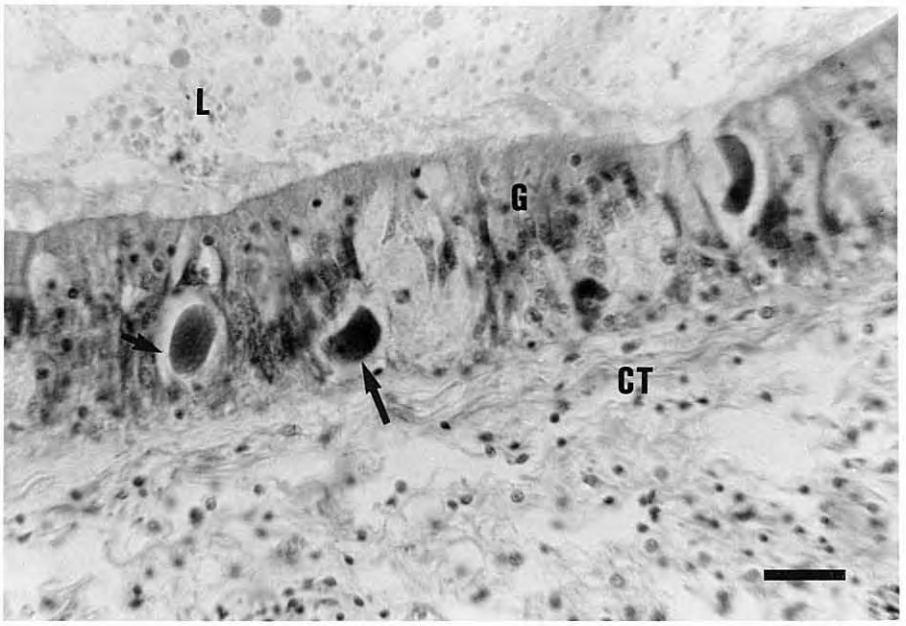


Fig 3.32: **Main gut epithelia (G) of *Crassostrea gigas* containing Type D rickettsial inclusions (large arrows). Note the lobed shape of the inclusions and the rarefaction of the surrounding cytoplasm (small arrow); CT = connective tissue; L = lumen (H & E). Scale bar = 20 μm.**

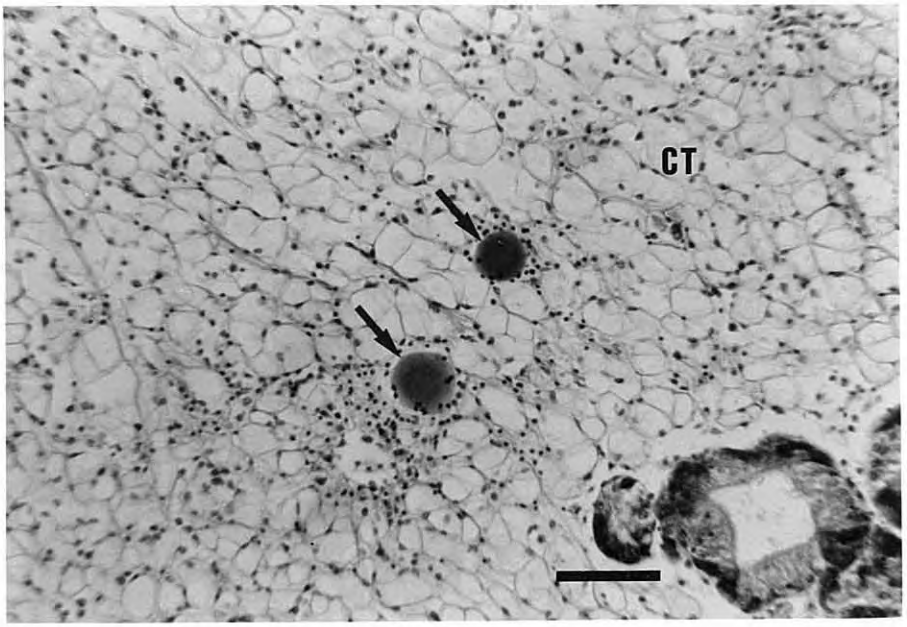


Fig 3.33: Connective tissue (CT) of *Crassostrea gigas* containing two Type E rickettsial inclusions (arrows) (H & E). Scale bar = 50μm.

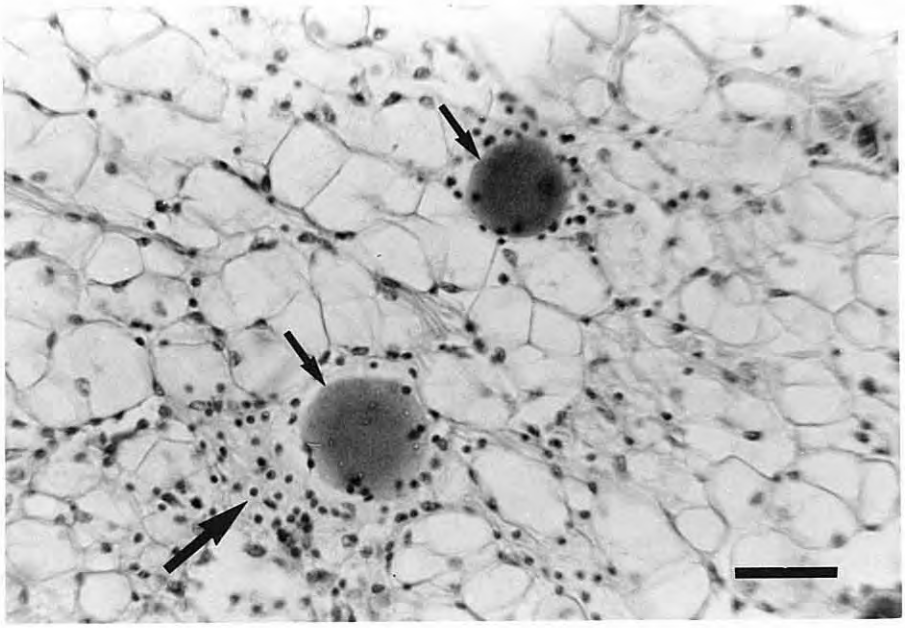


Fig 3.34: High power micrograph of a Type E rickettsial inclusions (small arrows) in *Crassostrea gigas* and associated slight haemocytic respone (large arrow) (H & E). Scale bar = 25μm.

consisting of a small number of haemocytes surrounding these inclusions was noted (Fig 3.34).

There was never more than one inclusion type detected in any single oyster. The host reaction to the inclusions consisted of a halo around the inclusions where the cellular material had been displaced or where the inclusion was contained within a vacuole. Cells were swollen due to the large size of the inclusions relative to the cell. A haemocytic reaction to the inclusions was only observed in response to Type E inclusions. Infected oysters appeared healthy and could not be differentiated from uninfected oysters by macroscopic examination.

Flat oyster inclusions

Flat oysters were infected with two morphological types of rickettsial inclusions. Both types of inclusion were intracytoplasmic, granular, basophilic and were located in the digestive tubule epithelia.

The larger type of inclusion (Type F) measured 38.1 µm (95% C.I. = 2.9 µm) x 29.7 µm (95% C.I. = 1.8 µm), varied in the degree of basophilia and was located in the non ciliated digestive epithelium (Fig 3.35). In some cases, these inclusions appeared to have a "lobed" or clefted structure with clear lines running through the centre (Fig 3.36) and were sometimes seen surrounded by a halo. Forty-two percent of flat oyster inclusions were of this type. Intensity of infection by Type F inclusions was sometimes very heavy with most digestive tubule profiles affected.

The second type (Type G) seen by light microscopy resembled the Type B inclusions described from Pacific oysters (Fig 3.37) in that they were smaller measuring 12.6 µm (C.I. = 3.1) x 18.4 µm (C.I. = 6.9 µm), were more basophilic, and located in the ciliated digestive epithelium and sometimes surrounded by a halo (Fig 3.38). These inclusions made up the remaining 58% of flat oyster inclusions. Infections of Type G inclusions were less intense sometimes with only one inclusion seen in an oyster section.

Whilst two morphological types of inclusions were observed histologically, causative organisms appeared identical when examined by electron microscopy.

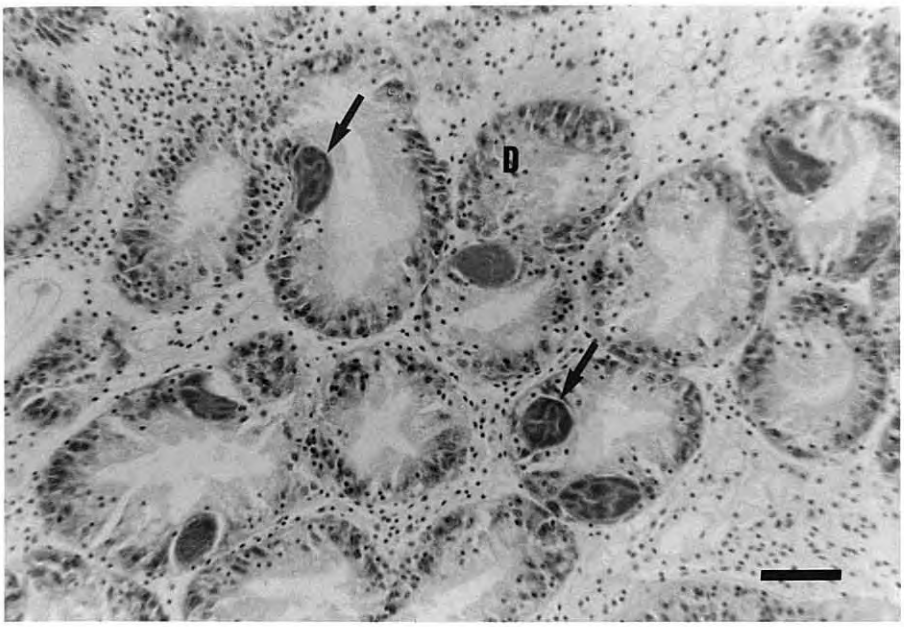


Fig 3.35: Type F rickettsial inclusions (arrows) in the digestive gland epithelia (D) of *Ostrea angasi* (H & E). Scale bar = 45 μm.

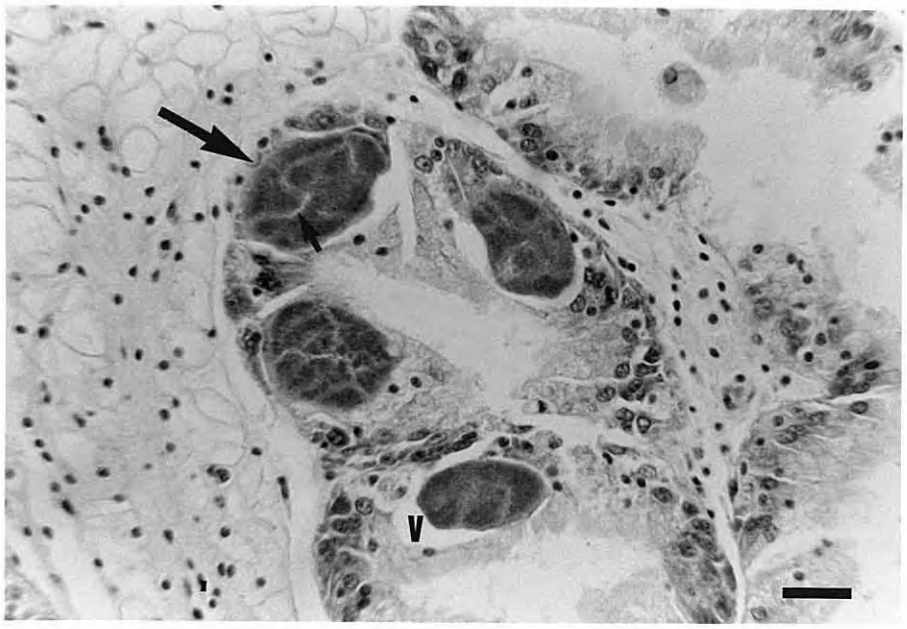


Fig 3.36: High power micrograph of Type F rickettsial inclusions in *Ostrea angasi* showing the clefted appearance (small arrow) of the inclusions (large arrow) and the location of the inclusions within vacuoles (V) (H & E). Scale bar = 20 μm.

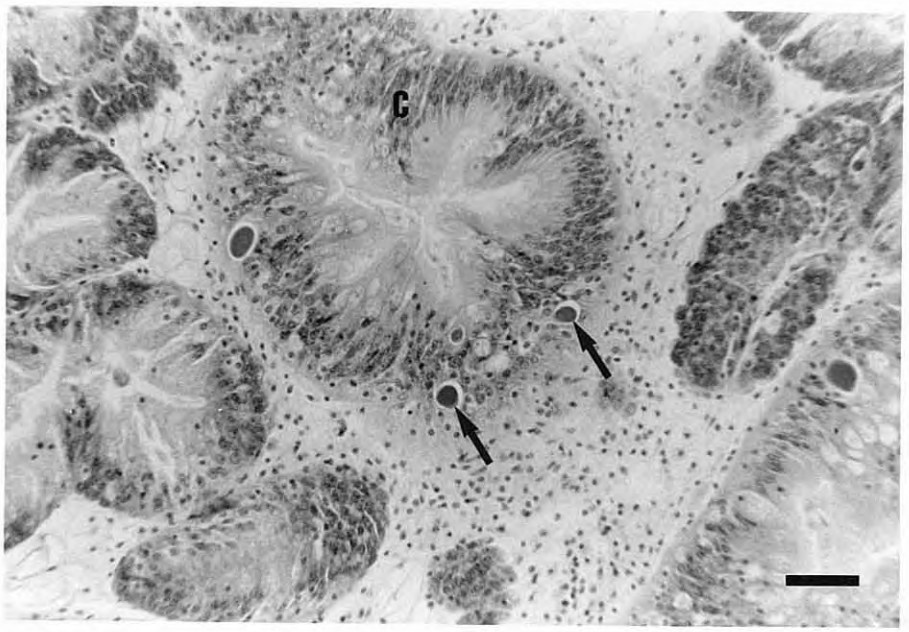


Fig 3.37: Ciliated digestive epithelia (C) of the digestive gland of *Ostrea angasi* containing Type G rickettsial inclusions (arrows) (H & E). Scale bar = 50μm.

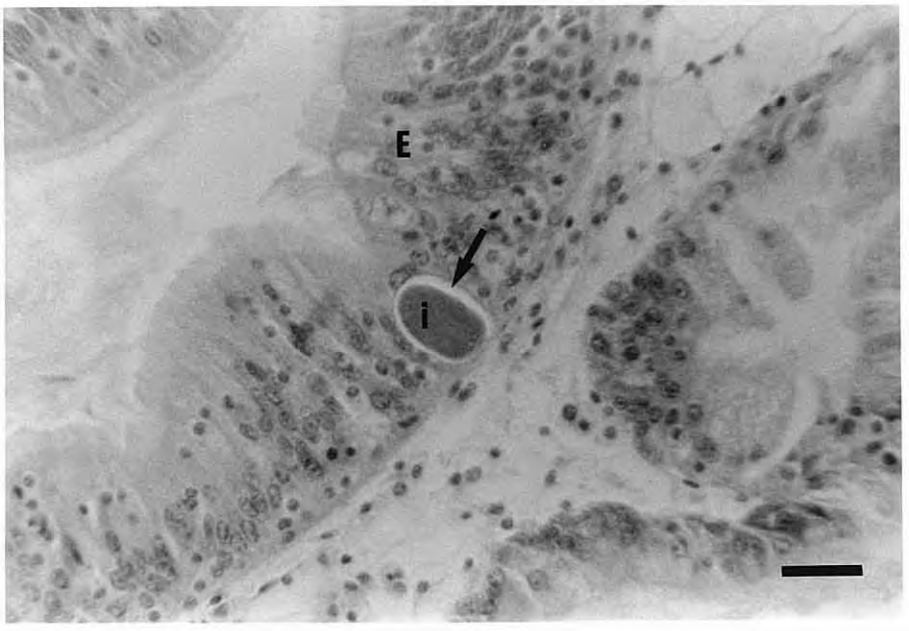


Fig 3.38: High power micrograph of Type G rickettsial inclusions (i) showing location within a vacuole (small arrow); E = ciliated digestive epithelium (H & E). Scale bar = 20μm.

Tissue containing Type F flat oyster inclusions, examined by electron microscopy had been deparrafinised from wax blocks. Type G inclusions examined by electron microscopy was obtained from glutaraldehyde-fixed tissue. When viewed by electron microscopy, both types of inclusions were bound by a double unit membrane, and contained prokaryotic organisms 200-300 nm in diameter. The organisms were pleomorphic and many appeared star shaped in cross section (Figs 3.39 and 3.40). It was thought at first that the star shape may have been an artifact of the rigorous procedure used during Davidson's fixation and deparrafinising. However, glutaraldehyde-fixed material showed the same shape in cross section. Some of the organisms contained electron dense patches resembling structures identified as condensing bodies characteristic of chlamydial organisms (Harshbarger et al., 1977). However, in the absence of any other corroborating evidence, these organisms were defined as rickettsial organisms.

Some inclusions had a double membrane running through the centre of the inclusion between rickettsial organisms (Fig 3.41). It may be that the clear lines or clefts observed histologically correspond to these membranes and represent a folding of the inclusion as it expands.

Electron microscopy studies also revealed that bodies resembling phages were present in some rickettsial organisms infecting *O. angasi*. Degeneration of the structure of the rickettsial organisms including the membranes was associated with the presence of the phages (Fig 3.42). These particles were regular in shape and measured 50 nm in diameter and were located throughout the body of the rickettsial organisms (Fig 3.43). It was observed that in an individual oyster, both phage infected and uninfected inclusions were present. However, where phages were present in an inclusion, most of the organisms within the inclusion were affected.

Histologically, there was no observable host response to the presence of the inclusions other than the halo or vacuole surrounding the inclusions, or where infected cells became swollen due to the large size of the inclusions. As with Pacific oysters, affected oysters appeared healthy and could not be differentiated from uninfected oysters by macroscopic examination.

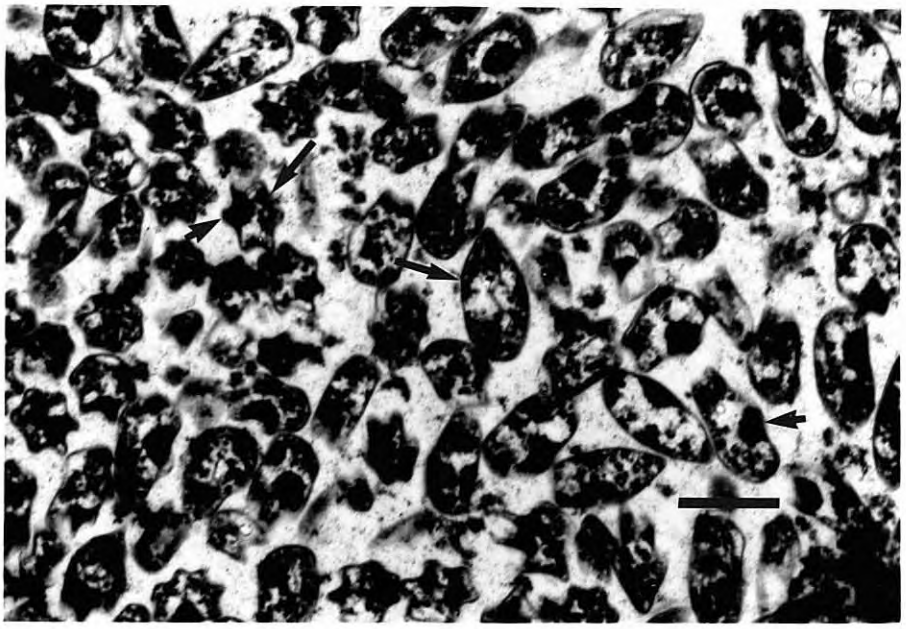


Fig 3.39: Electron micrograph of Type F rickettsial organisms (large arrows) in *Ostrea angasi*. Note some organisms contain electron dense patches (small arrows) and the pleomorphic nature of the organisms. Scale bar = 500 nm.

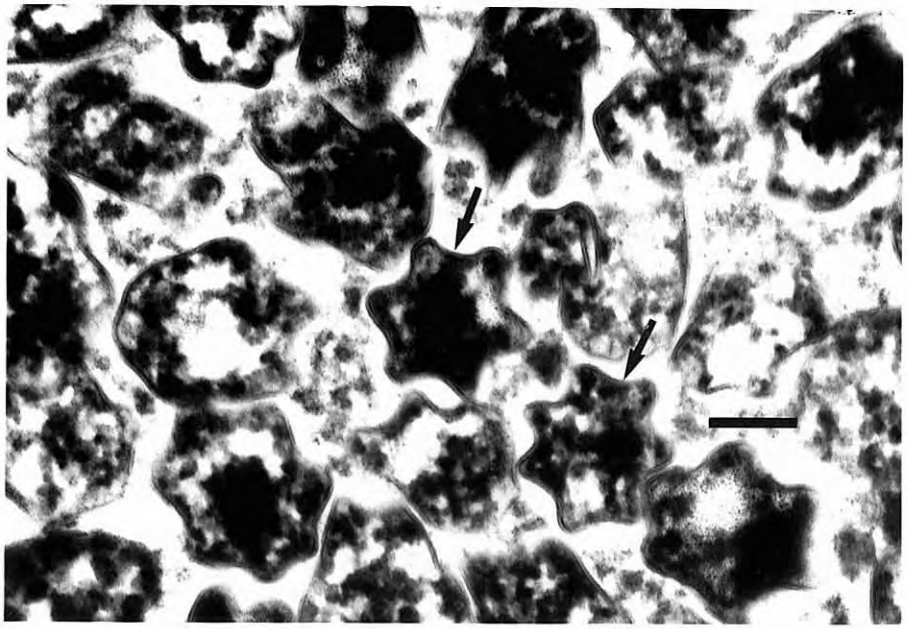


Fig 3.40: Electron micrograph of Type G rickettsial organisms in *Ostrea angasi* showing the star shape (arrows) of these organisms in cross section. Scale bar = 200 nm.

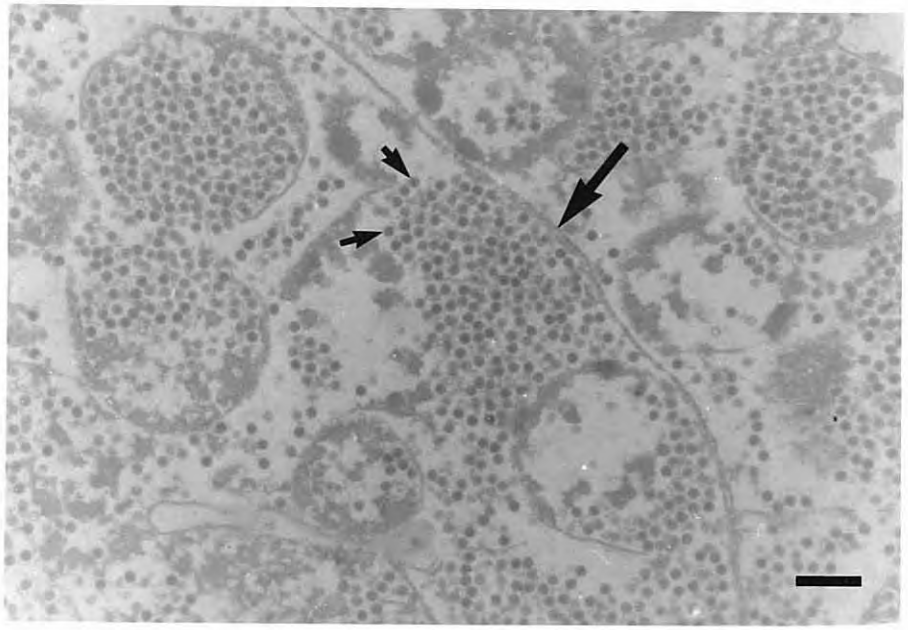


Fig 3.41: Electron micrograph of Type F rickettsial organisms in *Ostrea angasi* infected with phages (small arrows). Note the membrane (large arrow) running through the inclusion which may be the cause of the clefted structure seen in Fig 3.36. Scale bar = 300 nm.

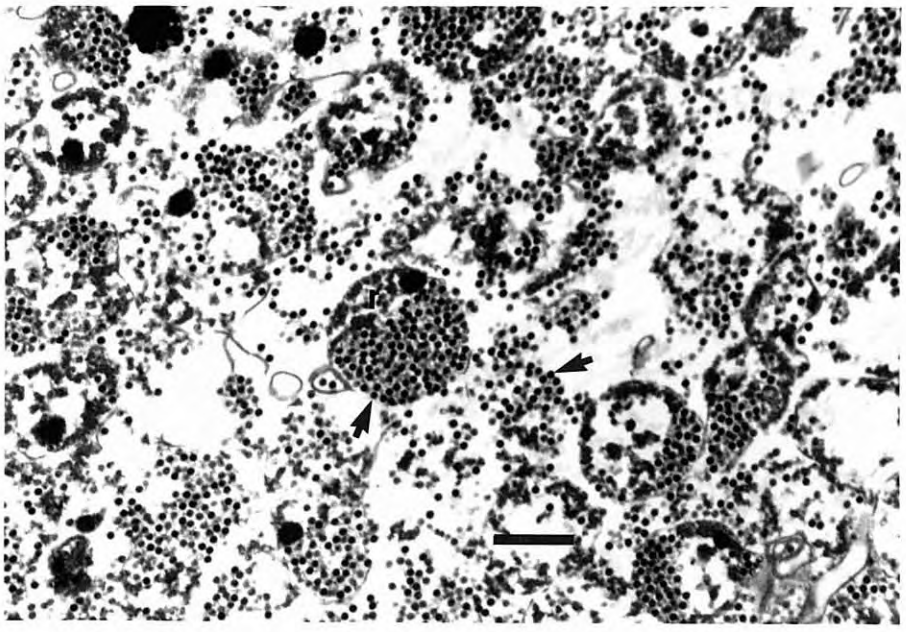


Fig 3.42: Phage particles (arrows) in Type F rickettsial organisms (r) in *Ostrea angasi*. Note the degenerate structure of the infected rickettsial organisms. Scale bar = 500nm.

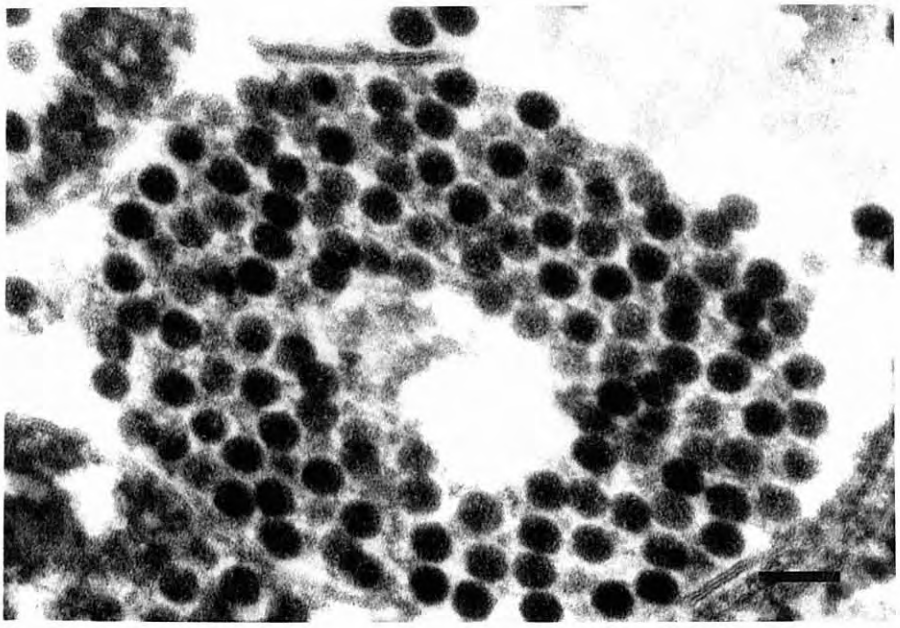


Fig 3.43: High magnification electron micrograph of phage particles in Type F rickettsial organisms. Note their regular size and shape. Scale bar = 100 nm.

Protozoa

Bonamia sp

Parasites consistent with the size and shape of *Bonamia* sp were found in the haemocytes of *O. angasi*. Parasites were circular, measured 2-3 µm in diameter with a 1 µm diameter nucleus and were located within a cytoplasmic vacuole within haemocytes (Fig 3.44). The cytoplasm of the parasite was pale staining with a darker nucleus. Bi-nucleate forms of *Bonamia* sp were observed.

Bonamia sp parasites were recognised in *O. angasi* in Tasmania by their size, shape, presence within a cytoplasmic vacuole within haemocytes and associated, sometimes severe, pathology. In addition, examination of reference histological material of *Bonamia ostreae* in *Ostrea edulis* and *Bonamia* sp from *O. angasi* in Victoria showed a similarity in morphology to the parasite seen in Tasmanian shellfish. Material was also examined by M. Hine who confirmed that the organism was a species of *Bonamia* and was probably *Bonamia* sp.

Bonamia sp parasites were not found in the samples prepared for electron microscopy by fixation in glutaraldehyde. Attempts to examine formalin-fixed material by electron microscopy had failed as the parasites collapse and artifacts are prevalent in this material.

The presence of *Bonamia* sp could not be determined by macroscopic observation. In the sample of 180 *O. angasi* collected on 14.4.92, the occurrence of an emaciated condition, eroded gills or pale digestive gland was not coincident with *Bonamia* sp infection (diagnosed by histological examination). However, from records of numbers of watery specimens in a sample and results of retrospective examinations of *O. angasi* for *Bonamia* sp, it was noted that oysters with a watery appearance often occurred in samples which were infected with *Bonamia* sp (Fig 3.11).

Histologically, the intensity of infections and associated haemocytic response in infected oysters varied from small foci of haemocytes around the gut or gill epithelium to diffuse haemocytosis in all tissues of the oyster with parasites not restricted to the epithelia.

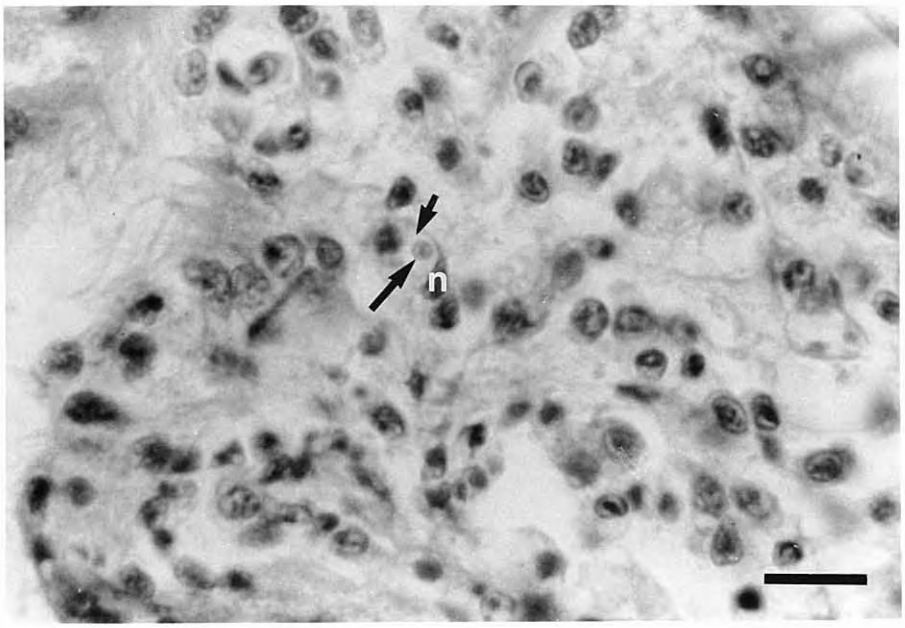


Fig 3.44: Single *Bonamia* sp parasite (large arrow) within cytoplasmic vacuole (small arrow) of haemocyte in the digestive epithelium; n = nucleus (H & E). Scale bar = 20μm.

A description of the scoring system used to rank the intensity is given below.

+S	Presence of small numbers of small lesions in the tissue but without parasites.
+E	Lesions as above but *Bonamia* sp parasites are present.
++M	One to several well developed focal lesions with *Bonamia* sp parasites present.
+++L	One to several large lesions including connective tissue surrounding gut or digestive gland
+++M	Excessive numbers of moderate lesions. Parasites are always present at this stage usually in the gut or digestive epithelium, but sometimes in the connective tissue.
++++	Severe lesions in the tissue and diffuse infiltrates of haemocytes throughout the tissue with parasites present in these diffuse infiltrates.
+++++	Severe diffuse haemocytic infiltrate with parasites extremely numerous in any part of the tissue.

In oysters from Georges Bay, the intensity of infection varied between +S and ++++ (Fig 3.45). The majority (82%) of infected oysters had *Bonamia* sp infections which scored +E or ++M.

In these infections, *Bonamia* sp parasites were difficult to find. Usually, only one *Bonamia* sp was seen per high power field and rarely were there more than one with a maximum of 3 parasites seen in an infected haemocyte (Fig 3.46). Parasites were never seen extracelluarly.

Where a focal lesion occurred in the digestive gland or gut, parasites were only found within the epithelium and not beyond the basal membrane, although haemocytic infiltration occurred both in the affected epithelium and adjacent connective tissue (Fig 3.47). Where these lesions occurred in the gill, parasites were often, but not exclusively, located in the gill epithelium and were also present in the subepithelial tissue (Fig 3.48). In all cases where infections occurred in the digestive gland, gut or gills, the haemocytic infiltration caused the epithelium to become swollen and distorted and in some cases, the basal membrane was displaced from the epithelial cells. It was not possible to determine whether the *Bonamia* sp parasites were located within the epithelial cells or the invading haemocytes.

All infected oysters collected from Georges Bay from January - November 1991 and the majority of those collected in April 1992, were scored as either +E or ++M indicating very low intensity infections.

Infections of higher intensity (++M, ++++) where large numbers of focal lesions or severe lesions with some diffuse haemocytic infiltrates were present, were observed only in samples collected on 14.4.92. This may have been due to the larger sample size collected at this time or the pattern of *Bonamia* sp infection where intensity of infection increases in autumn.

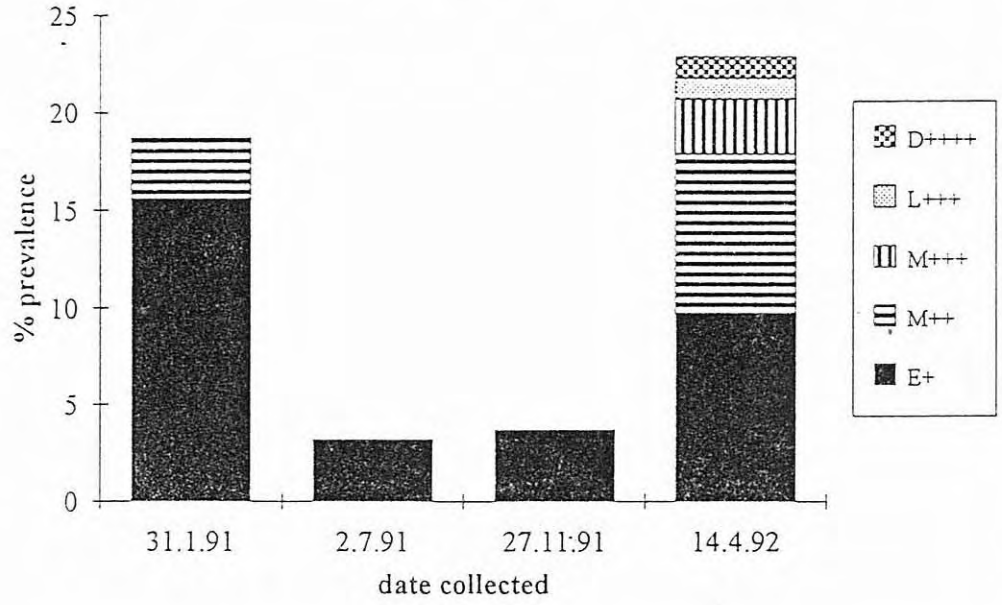
Even in these high intensity infections parasites were not abundant as described for infections of *Tiostrea chilensis* (Dinamani et al., 1987) and only up to 3 parasites per haemocyte were observed. The lesions and haemocytic infiltration was primarily located around the gut, digestive gland and gills.

The predominance of light infections with low numbers of parasites present within epithelial borders and association with focal haemocytic lesions is very different to the pathology associated with either *Bonamia* sp (Dinamani et al., 1987; Hine 1991a; Rawlins, pers. comm.) or *Bonamia ostreae* (Poder et al., 1982). *Bonamia* sp is usually associated with high numbers of parasites throughout the tissues and systemic haemocytic infiltration.

It was difficult to assess the effect of *Bonamia* sp on cultured stocks of shellfish in Tasmania. Low reported mortality in infected stocks and the predominance of focal haemocytic aggregates containing relatively few parasites may indicate that during the period of this study, *Bonamia* sp had little effect on the flat oyster populations studied. One lease (A) with *Bonamia* sp infected stock reported mortality up to 40% in stock held intertidally. This may be due to the intertidal habitat of *O. angasi* on this lease as *O. angasi* is intolerant of exposure to air for long periods (Anon, 1989).

The high levels of mortality and disease associated with this parasite elsewhere (Hine, 1991a; Rawlins, pers. comm.) indicate that this is, potentially, a very serious disease for flat oyster stocks in Tasmania.

Fig 3. 45: Prevalence and Intensity of Bonamia sp in O. angasi from Georges Bay.



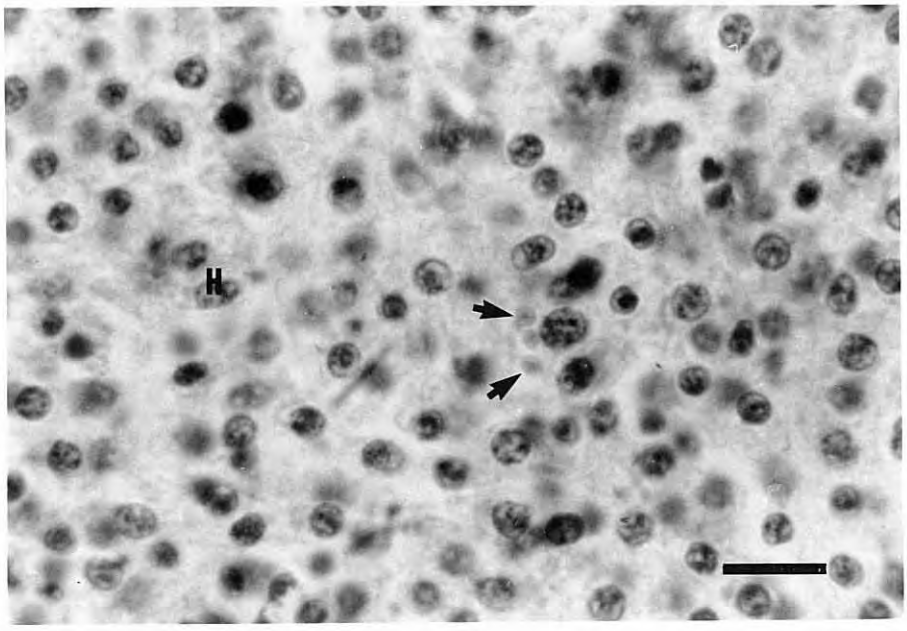


Fig 3.46: Three *Bonamia* sp parasites (arrows) within the cytoplasmic vacuole of one haemocyte in *Ostrea angasi*; H= uninfected haemocyte (H & E). Scale bar = 20μm.

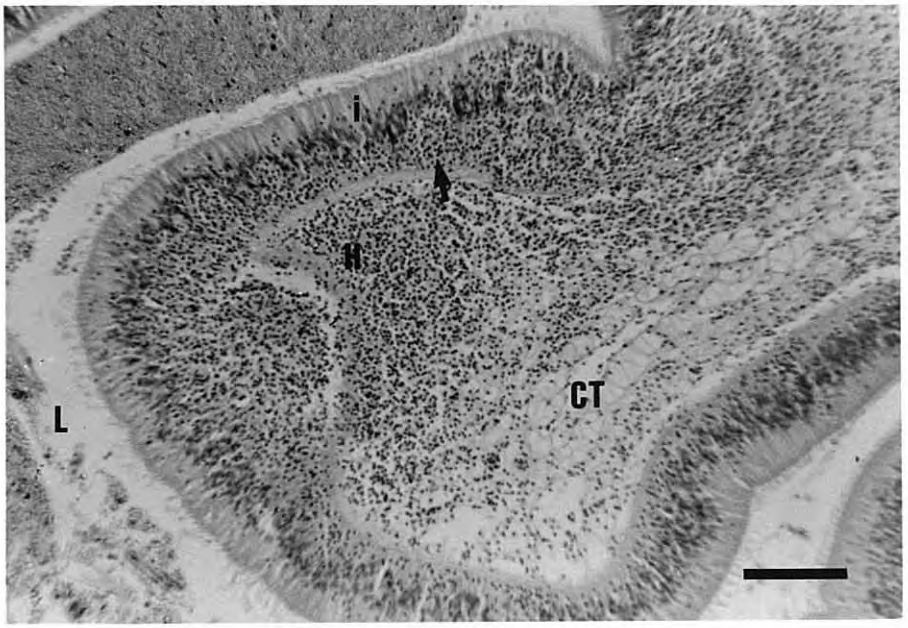


Fig 3.47 Epithelium of intestine (i) of *Ostrea angasi* infected with *Bonamia* sp showing local intense haemocytic infiltration (H). *Bonamia* sp parasites were found predominantly in haemocytes which had invaded the epithelium and rarely in haemocytes in the connective tissue (CT); L= lumen (H & E). Scale bar = 100μm.

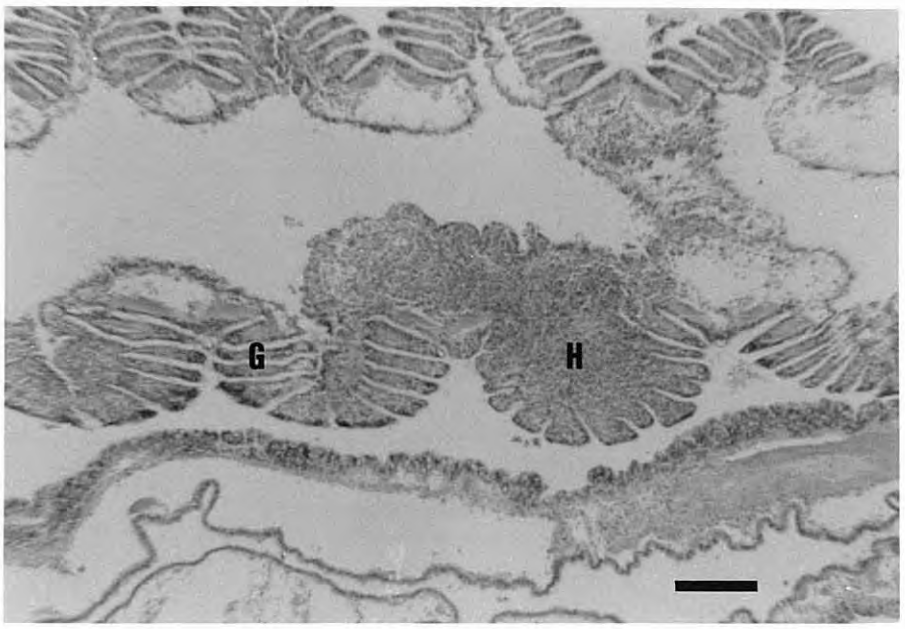


Fig 3.48: Gill filament (G) of *Ostrea angasi* infected with *Bonamia* sp showing local, intense haemocytic infiltration (H) (H & E). Scale bar = 200μm.

Ciliates

Ancistrocoma sp

Ciliates resembling *Ancistrocoma* sp were located in the lumen of the digestive tubules and were sometimes attached to the epithelium by the anterior end. These protozoans were pear shaped and measured 38.2 µm(95% C.I. = 11.1 µm) x 15.0 µm(95% C.I. = 3.9 µm) (Fig 3.49). In both species of oyster, the number of *Ancistrocoma* sp in a histological section was usually between 1 and 5, although in some oysters, heavier infections (up to 30 ciliates per section) were observed. There was no observable host pathology associated with the presence of these ciliates and they are not considered to be harmful to the health of either species of oyster.

Trichodina sp

Ciliates present on the surface of the gill and interlamellar epithelium (Fig 3.50) were recognised as *Trichodina* sp due to the presence of a horseshoe shaped nucleus and circular row of cilia on the underside of the organism (Fig 3.51). These ciliates measured 42.8 µm (C.I. = 2.8 µm) in diameter and 19.3 µm (C.I. = 3.0 µm) in height. They were often located regionally on the gill and on average, 2 were seen in a low power field. Infected oysters appeared healthy and aside from minor damage to the host at the point of attachment of the parasite, no pathology or host reaction was present.

Other protozoans

An additional two protozoans were found on the gills of Pacific oysters. Type A (Fig 3.52) was roughly ovoid shaped and measured 15.3 µm (95% C.I. = 2.0 µm) x 9.7 µm (95% C.I. = 1.7 µm) and had no cilia or flagella (Fig 3.53). Some were apparently attached to the gill epithelium by their narrowest end. Their position, size, shape, and method of attachment to the gill tissue was similar to that of *Sphenophyrea* sp (Cheng, 1988). *Sphenophyrea* sp is a species of ciliate which lacks cilia at the adult stage of its life cycle (Otto et al., 1979). Intensity of infections was low and hosts did not exhibit any cellular reaction to their presence.

In comparison, Type B organisms were rounded and larger than Type A, and were located between the epithelial cells of the gill (Fig 3.54).

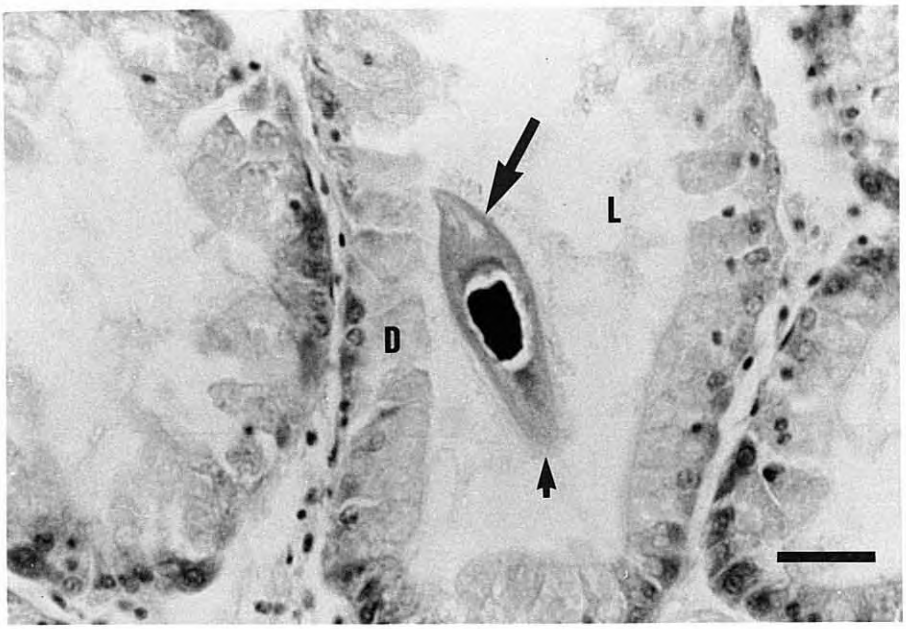


Fig 3.49: *Ancistrocoma* sp (arrow) in the lumen (L) of digestive tubules (D) in *Crassostrea gigas* (also found in *Ostrea angasi*) showing cilia (small arrow) (H & E). Scale bar = 20μm.

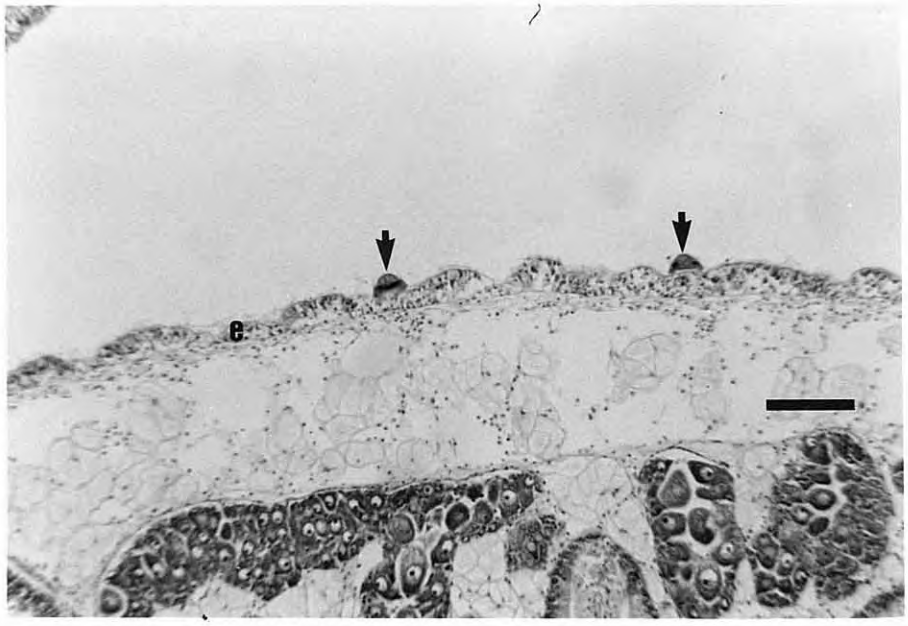


Fig 3.50: *Trichodina* sp (arrows) on the interlamellar epithelium (e) of *Crassostrea gigas* (H & E). Scale bar = 100μm.

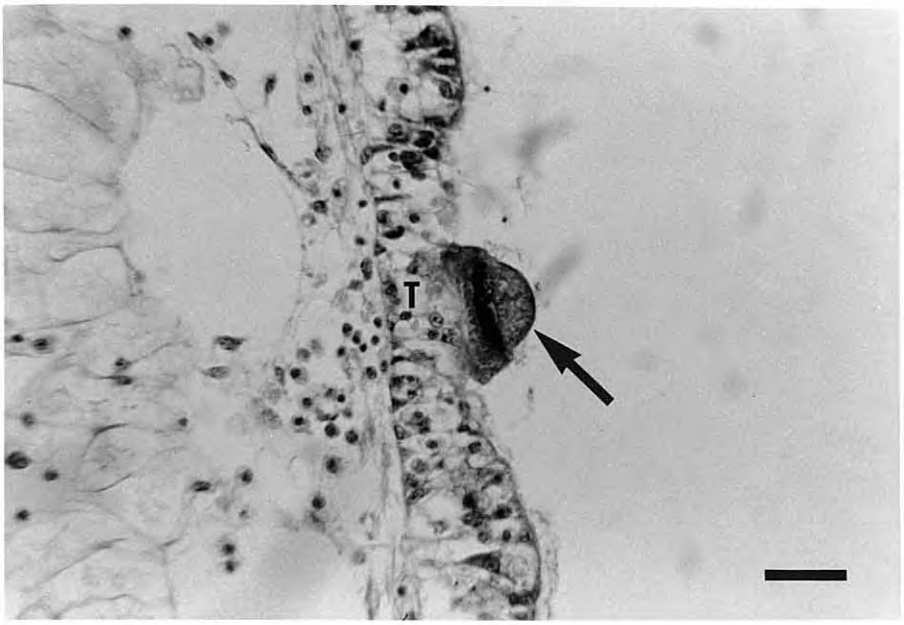


Fig 3.51: High power micrograph of *Trichodina* sp (arrow) from *Crassostrea gigas* showing thinning of epithelial cells (T) at the site of attachment (H & E). Scale bar = 25μm.

The organisms measured 22.7 µm (95% C>I. = 4.7 µm) x 14.6 µm (95% C.I. = 0.9 µm), and had a macronucleus and micronucleus typical of ciliates (Fig 3.55).

Neither of these showed any damage to the gill tissue and they were usually present in low numbers. Consequently, these organisms are also considered commensals.

Flat oysters were not infected with any of these gill organisms.

Metazoans

Turbellarians

Turbellarians were observed on rare occasions in the intestinal lumen of Pacific oysters (Fig 3.56). These organisms measured 77.9 µm (C.I. = 24.3 µm) in diameter, had a ciliated epithelium, a large sucker at the anterior end and dark patches on the body (eye spots). Some turbellarians were attached to the intestinal epithelium by the sucker with host material being engulfed (Fig 3.57). It is unclear whether these turbellarians are consuming host tissue or using the sucker as a means of attachment. Some damage to the intestinal epithelium in the region of attachment was noted and included thinning of the epithelium and moderate infiltration of haemocytes to the infected area (Fig 3.56). Up to 4 organisms were seen in a low power field.

The low prevalence and intensity of these infections and the small amount of localised damage associated with these organisms indicates they are not of significance to Pacific oyster health.

Polychaetes

The three species of spionid polychaetes seen in shellblisters were also found on the shell surface and in the mantle fluid. It is possible that some or all of these (especially spionid polychaetes) had moved there from shellblisters during transportation. The majority of "external" polychaetes were recovered from the shell surface. Only specimens from the families Spionidae, Nereididae and Terebellidae were found in the mantle fluid.

Data on the size, thickness and contents of shellblisters of *Crassostrea gigas* was recorded for the period April to July 1991.

Fig 3.52: Gill organism Type A (*Sphenophyra* sp?) (arrows) on gill filament (G) of *Crassotrea gigas* (H & E). Scale bar = 50 μm.

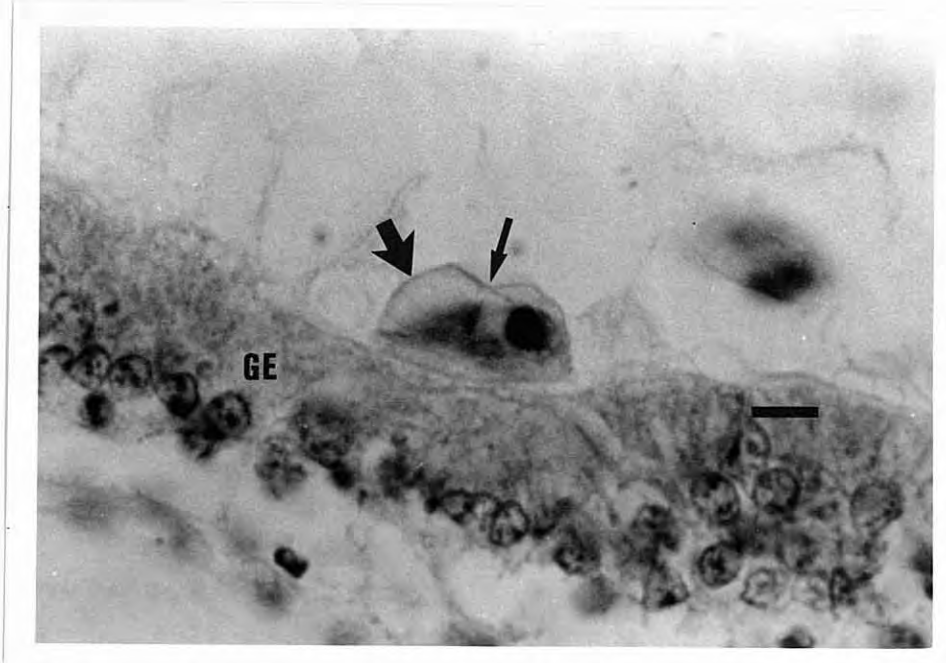


Fig 3.53: High power micrograph of gill organism Type A (wide arrow) on gill epithelium (GE) of *Crassostrea gigas* showing the crenulated membrane (small arrow), and apparent lack of cilia (H & E). Scale bar = 5μm.

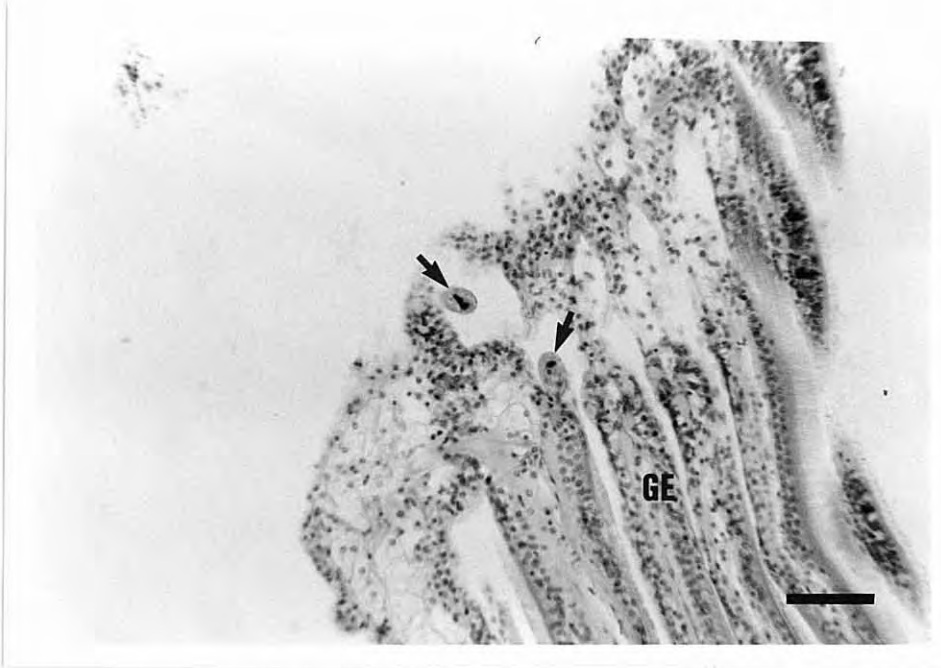


Fig 3.54: Gill organism Type B (arrows) associated with the gill epithelium (GE) of *Crassostrea gigas*. Note their position between the secondary lamellae (H & E). Scale bar = 50μm.

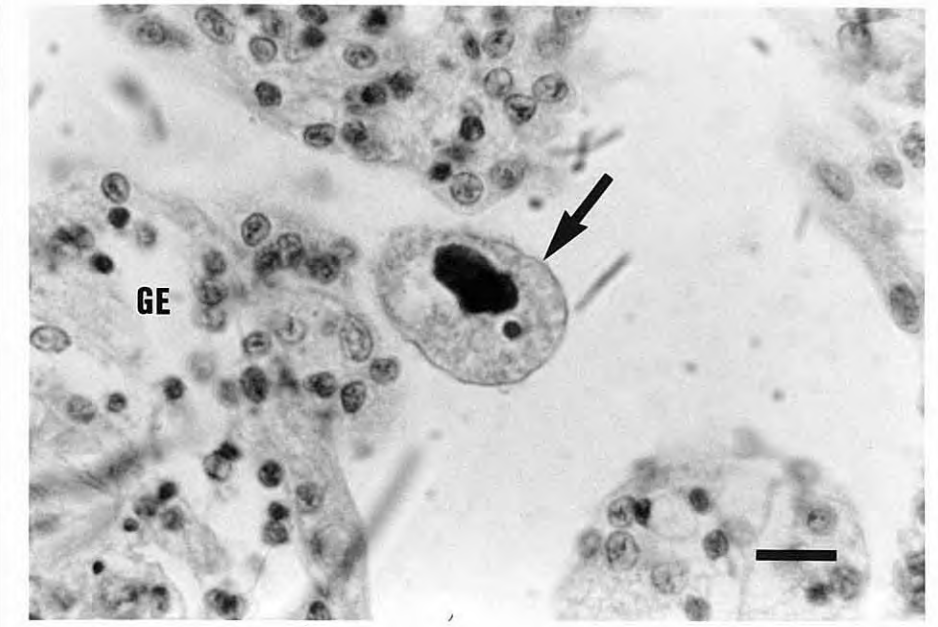


Fig 3.55: High power micrograph of Type B gill organism attached to the gill epithelium (GE) showing the rounded shape and apparent lack of cilia (H & E). Scale bar = 15μm.

Fig 3.56: Turbellarians (arrows) in the lumen of the intestine of *Crassostrea gigas*. Note the localised thinning (small arrow) of the digestive gland epithelium near the site of attachment (H & E). Scale bar = 200μm.

Fig 3.57: Turbellarians in the lumen of the intestine of *Crassostrea gigas*. Note the attachment of the turbellarian to the epithelium and the associated engulfing of host material (E), the ciliated epithelium (thin arrow), and the dark patches (eye spots) on the body (wide arrow) (H & E). Scale bar = 30μm.

Fig 3.58: Pacific oyster shell with thin shelled shellblister (arrow) containing mud.

Polychaetes causing the shellblisters were always isolated from the meat of the oyster by a layer of shell which formed the shellblister. The presence of the shellblisters was only rarely associated with any lesion or damage to the meat.

The size of the blisters was highly variable between 3-15 mm wide and 5-25 mm in length. On rare occasions the blister was large and covered up to 70% of the valve.

Shellblisters were found on both valves although they were more common on the upper valve (72.3%) compared with the lower valve (27.7%).

The thickness of the shell of the blister varied between a thin membrane or shell (Fig 3.58), a moderately thick shell which could be broken with a scalpel blade or a very thick shell which was difficult to break. These very thick shells were white compared with a brown appearance of the thinner blisters, and were almost indistinguishable from the oyster shell. In Pacific oysters, 46% of the blisters had thin shells and 54% were moderate or thick shells. Blisters infected with *P. websteri* were more often thin shelled with 65% of *P. websteri* infected blisters with thin shells.

The majority of the blisters (75%) contained mud with the remaining 25% containing only seawater and faecal material.

Pseudomyicola spinosus

Crustaceans resembling *Pseudomyicola spinosus* were found in the contents of the gut and invading the tissues of the digestive diverticula and mantle of both Pacific and flat oysters. The parasites measured approximately 297.4 μm (95% C.I. = 64.1 μm) and in most cases a carapace with jointed appendages could be seen. Where the copepod was entirely within the contents of the gut the oyster showed no response to the parasite and the internal structure of the parasite appeared intact (Fig 3.59,3.60). In cases where it had moved through the digestive gland lining or invaded the connective tissue (Fig 3.61), it was surrounded by an intense focus of haemocytes and the epithelial tissue was disorganized and or disrupted (Fig 3.62). In all of these cases the internal structure of the copepod was degenerate and disorganized indicating successful destruction of the parasite by the oyster. Usually only 1-2 copepods were observed per section, although in some cases up to 5 were observed.

These organisms were found in apparently healthy shellfish and are considered commensal in nature.

Gill copepods

Copepods were also observed invading the gill tissue of Pacific and flat oysters where they elicited a pronounced local haemocytic response (Fig 3.63). The infiltrating haemocytes were noted to have a large proportion of cytoplasm and appeared foamy. Because of the degenerate state of these specimens, identification further than a crustacean and probably copepod was not possible. These organisms were recognised as crustaceans by the remnant exoskeleton and short bands of muscle fibres remaining in the degenerate body. It is not known whether these are the same species as copepods found in the gut.

Abnormal conditions

Histological examination did not reveal a cause for the watery condition observed in Pacific oysters. In a number of cases, nuclei with marginated chromatin were seen, which may indicate a previous viral infection. However, as inclusions were not observed this cannot be confirmed.

3.3 Changes in the histological appearance of Pacific oysters.

X^2 analysis of relative proportions of scores for connective tissue oedema (LEY0, LEY1+2), diffuse haemocytic infiltration (HC0, HC1+2D), focal haemocytic infiltration (HC 0, HC 2+3L), and brown cell abundance (BWN 0, BWN 1+2), showed that significant differences occurred between areas, and significant differences occurred between seasons for each area.

Unusually high or low levels of scores for particular tissue changes (eg. connective tissue oedema) were identified by examination of contributions of each X^2 cell to the overall X^2 value. The occurrence during the sampling period of these high or low scores for LEY 0, HC 2+3L, HC 1+2D, BWN 1+2 is shown in Figure 3.64. The variation in numbers of oysters with a proportion of their digestive gland experiencing digestive tubule atrophy is shown in Fig 3.65. In many cases, there were consistent seasonal trends in the pattern of tissue changes across most or all areas.

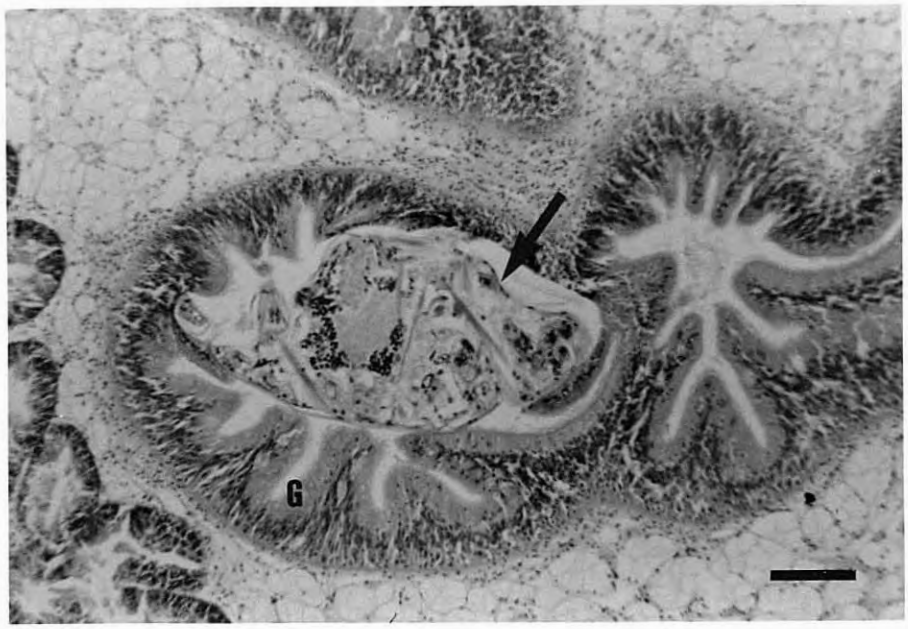


Fig 3.59: *Pseudomyicola spinosus* (arrow) in the lumen of the gut (G) of *Crassostrea gigas* (H & E). Scale bar = 75μm.

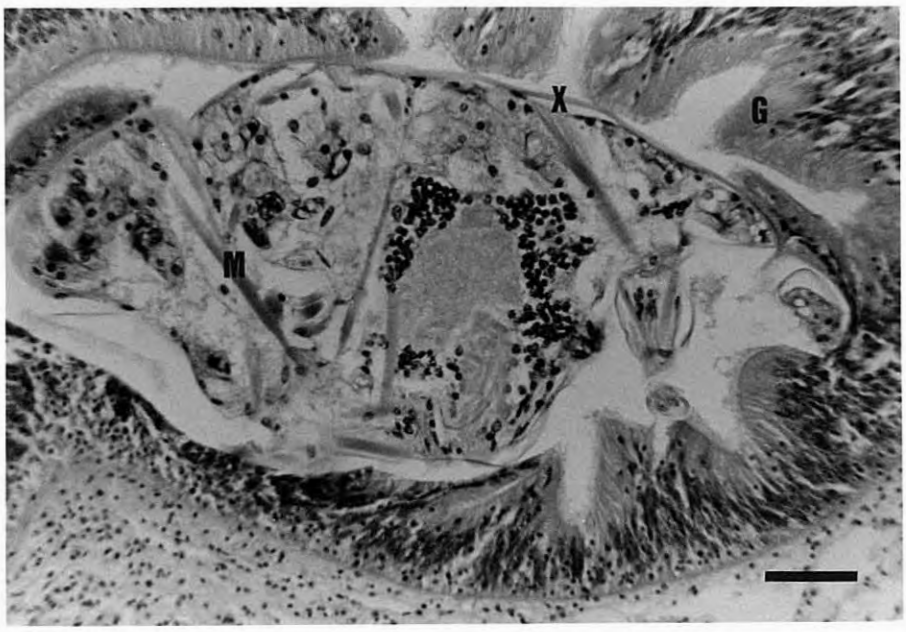


Fig 3.60: High power micrograph of *Pseudomyicola spinosus* (arrows) in the lumen of the gut (G) showing the internal structure of the copepod is intact; exoskeleton (X); muscle bands (M) (H & E). Scale bar = 50μm.

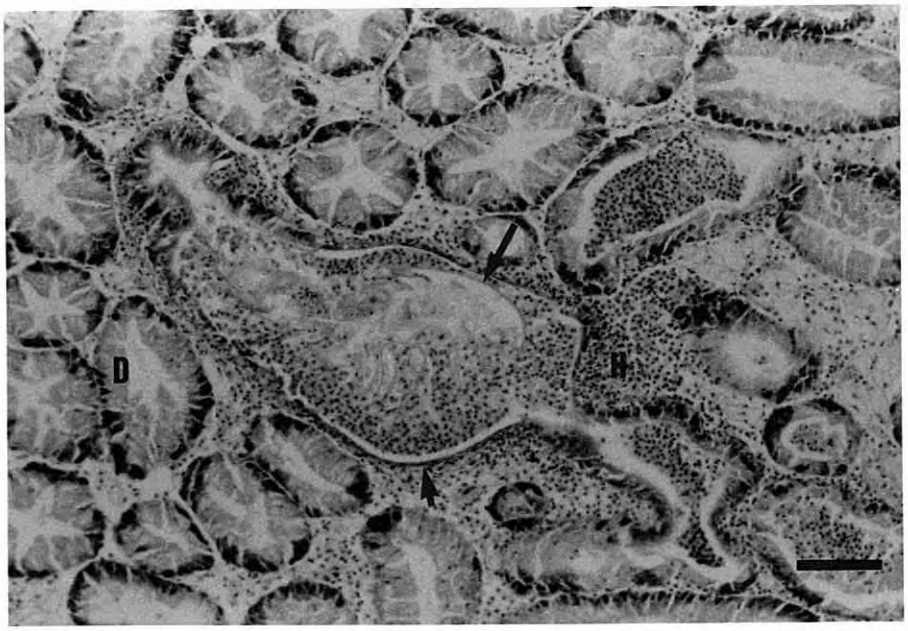


Fig 3.61: *Pseudomyicola spinosus* (large arrow) invading the digestive epithelium (D) of *Crassostrea gigas*. Note the thinning of the digestive epithelia (small arrow) and the localised haemocytic infiltration (H) (H & E). Scale bar = 100μm.

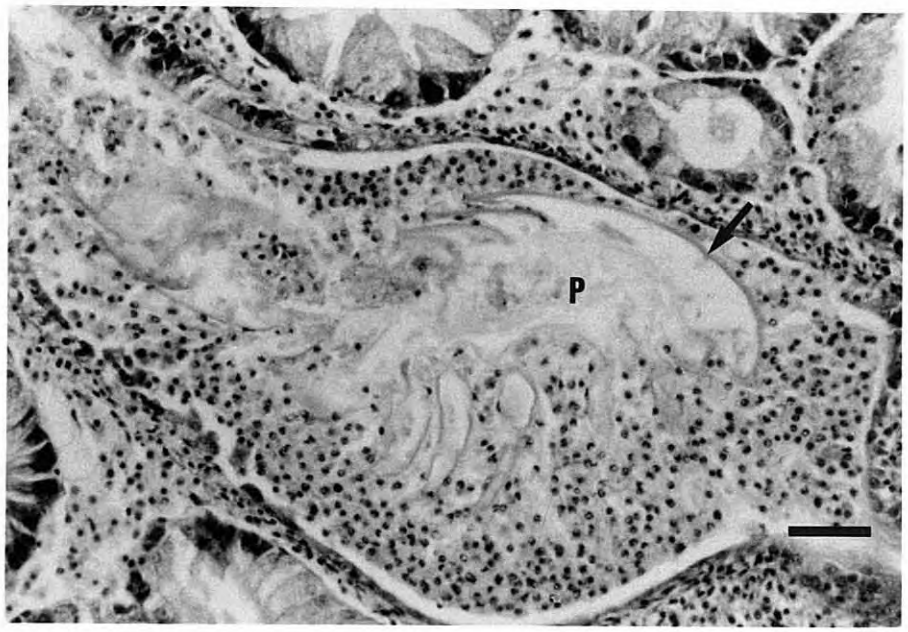


Fig 3.62: High power micrograph of *Pseudomyicola spinosus* (P) invading the digestive epithelia showing the degeneration of the internal structure of the copepod with only the exoskeleton (arrow) remaining (H & E). Scale bar = 30μm.

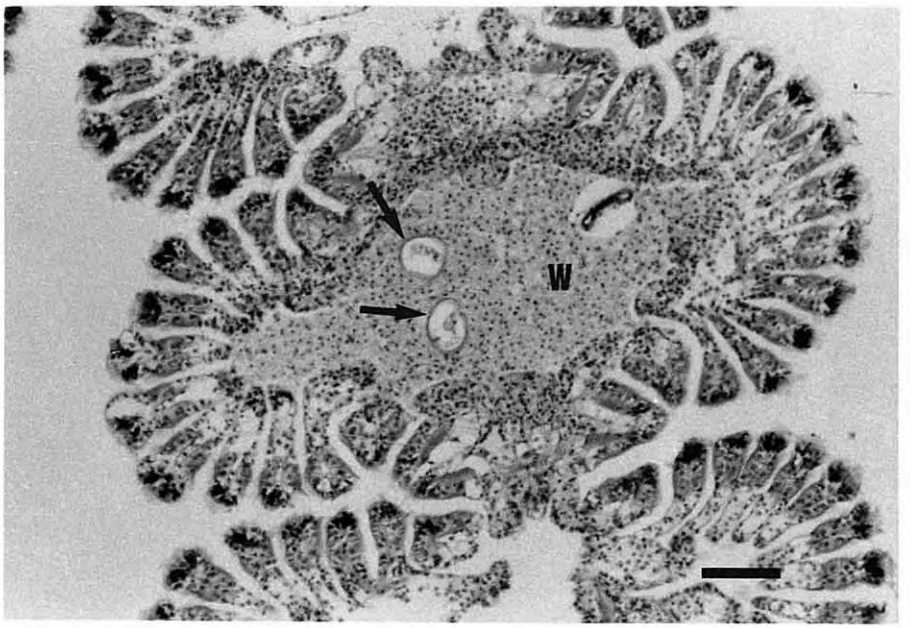


Fig 3.63: A small crustacean (arrows) (probably a copepod) invading the water tubules (W) of the gill of *Crassostrea gigas*. Note the intense localised haemocytic infiltration and the degenerate structure of the copepod indicating successful destruction by the host (H & E). Scale bar = 60μm.

Temperature and salinity data corresponding to the time of oyster sampling was available for 26 samples (out of a total of 58 samples). Of these, 8 were from Area 1, 5 from Area 2, 8 from Area 3, and 5 from Area 4. In all areas, data for at least one sample in each season was available minimising the effect of seasonal variation.

The results of parametric correlation analysis of the occurrence of tissue changes in relation to stage of gonadal development, environmental factors and in relation to the occurrence of other tissue changes is shown in Table 3.9.

Values of r shown in bold were statistically significant at 5% level of significance.

In this section, the pattern of tissue changes determined by these two types of analyses are described.

High levels of minimal tissue oedema occurred at apparently random intervals throughout the year (Fig 3.64). However, levels of minimal tissue oedema (LEY 0) were high in the east coast, south east and Channel region during October 1990 and in all areas during September-October 1991. In 1991, this was followed by high levels of connective tissue oedema in January and February 1992, and in the south east, this condition persisted until April 1992. This strong seasonal trend seen in September-February in 1991-1992, occurred concurrently with gonad development with connective tissue oedema at a minimum before spawning and increasing at or after spawning.

Although low levels of connective tissue oedema (LEY 0) was not significantly correlated with gonadal stage ($r=0.18$ (GONAD <4), $r=-0.33$ (GONAD >4)), this may be due to the large variation in the pattern of connective tissue edema between areas at other times of the year.

Levels of localised haemocytic infiltration were high on the northwest coast during January and February 1992 and on the east coast during February 1991 and January and February 1992. In the south east elevated levels were only seen during April, 1992. Increased localised haemocytic infiltration was positively correlated with occurrence of post spawned oysters (GONAD >4) ($r=0.59$). The correlation was stronger for occurrence of regressed gonads (GONAD R) ($r=0.73$). This would indicate that focal infiltration of haemocytes is related to gonad development in Pacific oysters.

Fig 3.64: Graphical representation of unusually high (▲) or low (▼) numbers of oysters with tissue scores representative of Pacific oyster health

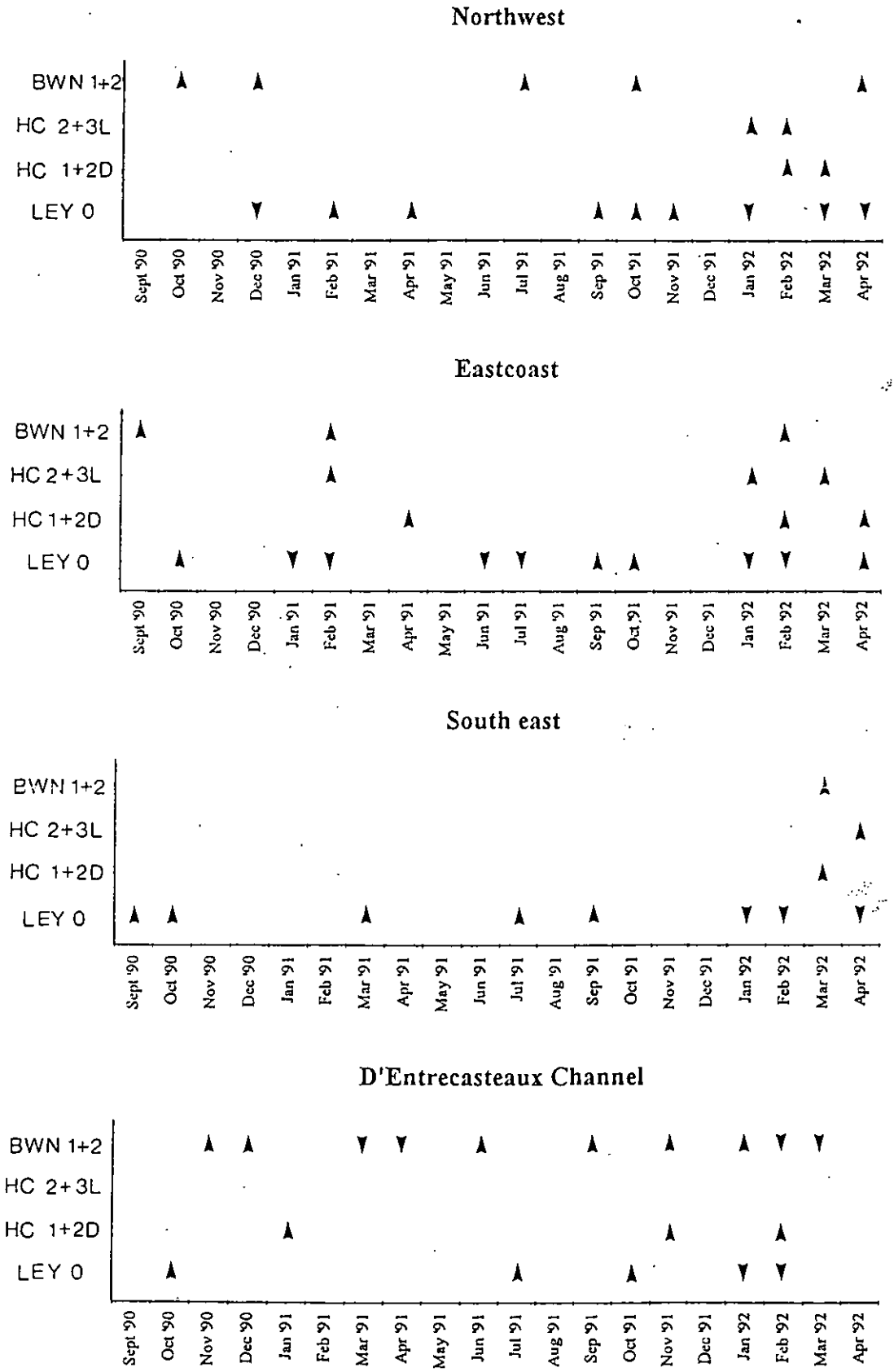
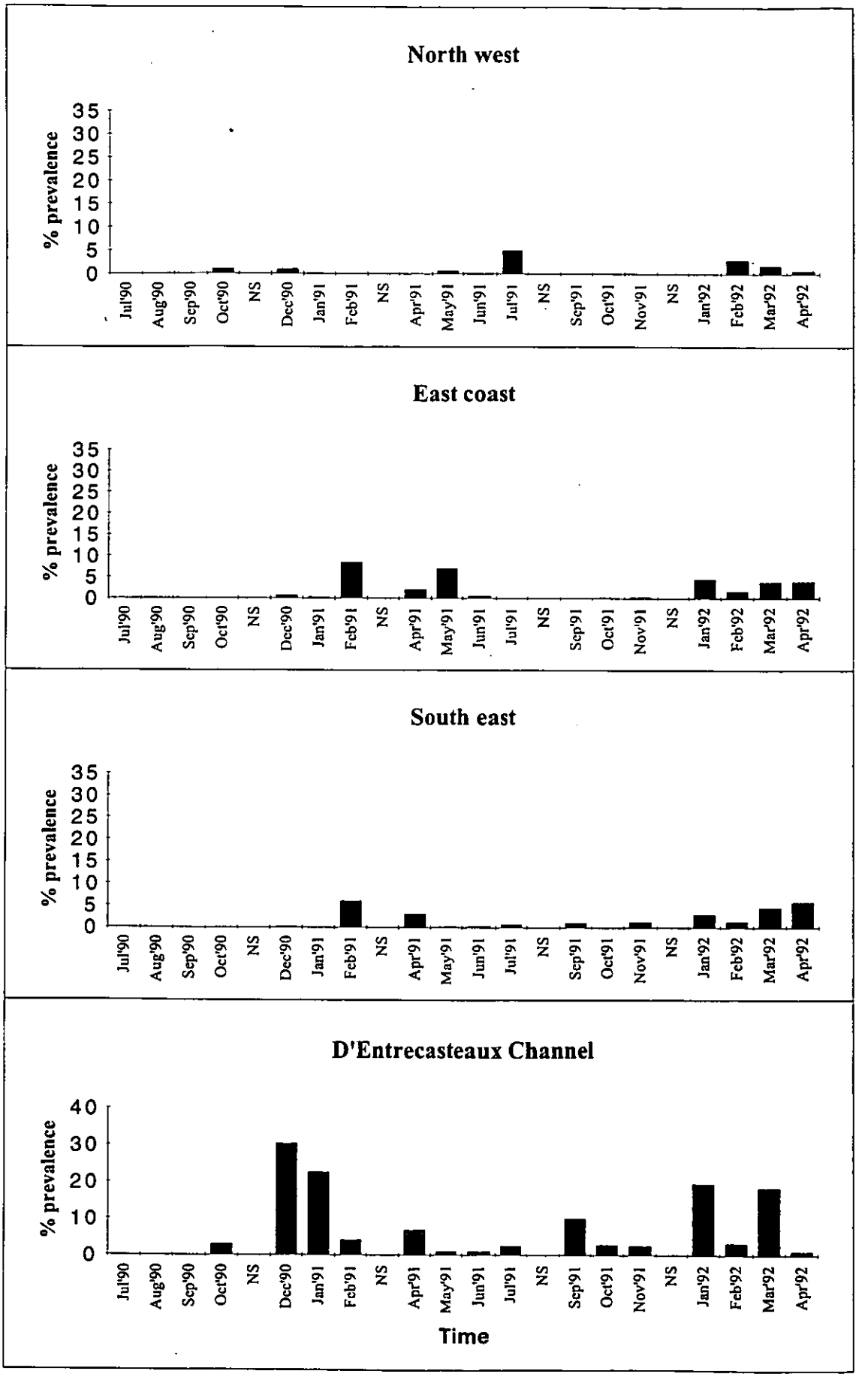


Fig 3.65: Prevalence of Pacific oysters with scores of digestive tubule atrophy (DTA) 1 or 2.



NS = no sample collected for this month

High levels of increased diffuse infiltration of haemocytes occurred during February or March 1992 in all areas, again at the time when most oysters have spawned. In addition, on the east coast, high levels occurred in April 1991 and April 1992.

Similarly to connective tissue oedema, diffuse haemocytic infiltration was not significantly correlated with stage of gonad development. Again, this may be due to variations in local condition at other times of the year.

Increased brown cell abundance and increase in digestive tubule atrophy occurred predominantly in the D'Entrecasteaux Channel (Figures 3.64 and 3.65) respectively. There were no consistent seasonal trends across in the pattern of brown cell abundance in this or any other area. Rather, the pattern of brown cell abundance was influenced by environmental conditions. Correlation analysis showed that increased brown cell abundance (BWN 1+2) was negatively correlated with salinity (SAL) (r=-0.54).

Increased digestive tubule atrophy (DTA 1+2) was also negatively correlated with salinity (SAL) (r=0.45) i.e. at low salinities the height of digestive tubule epithelium decreases. In addition, seasonal variations in epithelial height were related to the stage of gonadal development. In prespawning oysters (GONAD <4) digestive tubule atrophy was at a minumum (DTA 0) (r=0.49), but increased digestive tubule atrophy (DTA 1+2) was apparent in spawning and post-spawning oysters (GONAD<4) (r=0.55)

It was also apparent from the correlation analysis (Table 3.9) that the occurrence of a particular tissue condition was significantly correlated with the occurrence of other tissue conditions.

There was a positive correlation between minimal connective tissue oedema (LEY 0) and minimal digestive tubule atrophy (DTA 0) (r=0.5) and an inverse relationship between connective tissue oedema and increased diffuse haemocytic infiltration (HC 1+2D).

Levels of localised haemocytic infiltration (HC 2+3L) varied directly with increased brown cell abundance (BWN 1+2) (r=0.5) and degree of digestive tubule atrophy (DTA 1+2) (r=0.4).

Table 3. 9: Correlation table for tissue conditions scores, environmental conditions and stage of gonad development. Values in bold represent significant correlation between variables.

	TEMP	*SAL*	*LEY 0*	*HC 2+3L*	*HC 2+3D*	*BWN 0*	*BWN 1+2*	*DTA 0*	*DTA 1+ 2*	*G <4*	*G >4*	*G R*
TEMP	1											
SAL	**0.41**	1										
LEY 0	-0.21	0.18	1									
HC 2+3L	0.02	-0.09	-0.34	1								
HC 2+3D	-0.14	-0.23	**-0.39**	0.30	1							
BWN 0	0.28	**0.54**	0.34	**-0.47**	-0.30	1						
BWN 1+2	-0.28	**-0.54**	-0.34	**0.47**	0.30	-1.00	1					
DTA 0	0.20	**0.45**	**0.51**	**-0.39**	-0.26	**0.43**	**-0.43**	1				
DTA 1+ 2	-0.20	**-0.45**	**-0.51**	**0.39**	0.26	**-0.42**	**0.42**	-1.00	1			
G <4	-0.22	-0.01	0.18	**-0.47**	-0.22	0.08	-0.08	**0.49**	**-0.49**	1		
G >4	0.19	-0.04	-0.33	**0.59**	0.27	-0.18	0.18	**-0.59**	**0.60**	-0.87	1	
G R	0.11	-0.05	-0.32	**0.73**	0.25	-0.32	0.31	-0.28	0.28	-0.61	0.70	1

Correspondingly, increased brown cell abundance (BWN 1+2) was positively correlated with degree of digestive tubule atrophy (DTA 1+2)($r = 0.42$).

This analysis indicated that conditions considered to indicate good general health (low numbers of brown cells, non-atrophy of digestive epithelium, "full" connective tissue and absence of haemocytic infiltration) occurred concurrently. Also, conditions considered to indicate poor general health (increased numbers of brown cells, atrophy of digestive epithelium, connective tissue oedema and focal accumulation of haemocytes) occurred concurrently.

CHAPTER 4. DISCUSSION

This chapter considers the health of farmed oyster stocks in Tasmania with emphasis on *Crassostrea gigas* (Pacific oyster) stocks. The prevalence, temporal and geographic distribution, intensity, and effect on the host of commensal and parasitic organisms associated with Pacific oysters (*Crassostrea gigas*) and flat oysters (*Ostrea angasi*) are discussed. Changes in the histological appearance of Pacific oyster tissues are discussed in relation to time of year, gonadal development and environmental conditions.

Also, the significance of these findings for the Tasmanian oyster industry are given.

Commensals, parasites and diseases

Viruses

Ovacystis

Ovacystis infection was found in relatively low numbers of *C. gigas* (<1%) during this study. Prevalences of infection between 1 and 30% have been reported by Farley (1985) in crassostreid and ostreid oyster species. Ovacystis infections are ubiquitous in oyster populations world-wide and are recorded from *Crassostrea virginica* along the eastern seaboard of the United States, *C. rhizophorae* in Puerto Rico, *Ostrea lurida* in USA, Korea and Japan and *C. gigas* in USA, Korea and Japan.

The identification of this infection in *C. gigas* in Tasmania reflects the widespread distribution of this virus which may have been spread with movements of oysters between countries.

The observations of this study that infections occur mainly in females, that gametes are infected during the maturing stages of gametogenesis and that low numbers of gametes per oyster were infected, are consistent with the findings of Farley (1976; 1985).

The high proportion of infected gametes in post spawned oysters may indicate that higher rates of infection occur in oysters stressed by spawning or, that infected gametes are not released during spawning.

The observation of papovavirus particles in the nucleus of ovacystis infected cells is consistent with the findings of Farley (1976).

While ovacystis infections have not been associated with disease or mortality, Farley (1985) noted that papovaviruses have oncogenic properties and the effect of papovavirus infection on other species of shellfish or on populations of oysters previously unexposed to the infection, is unknown. For these reasons Farley (1985) suggested that movements of oysters between infected and uninfected areas and countries should be restricted.

It is not necessary to restrict movements of oysters in Tasmania due to the widespread distribution of infections in farmed oyster populations. In Tasmania, infections occurred in healthy oysters and were not associated with disease or mortality.

Virus X

The occurrence of intranuclear inclusions (probably caused by a herpes virus) in a single flat oyster indicates that the prevalence of this infection in farmed flat oysters in Tasmania is very low.

In the one infected oyster studied, massive haemocytic infiltration and cellular damage associated with the inclusions may eventually have caused the oyster to die. The death of low numbers of oysters with these infections may be attributed to "background" mortality as increased mortality levels in affected populations was not reported.

Herpes-like viral infections have previously been reported from *C. virginica* in USA (Farley et al., 1972) and from larval *C. gigas* in New Zealand (Hine et al., 1992) and France (Nicolas et al., 1992). In these studies, mortality of infected oysters occurred in water of elevated temperature. Farley et al. (1972) noted that in *C. virginica* an infection which caused 50% mortality in a population exposed to high water temperatures was inapparent at ambient water temperatures.

In this study, temperature may have influenced the expression of this disease as inclusions were found in an oyster collected during summer from the east coast (an area of higher water temperatures than other regions of Tasmania). The low prevalence of infection and apparent minimal effect of this viral infection in *O. angasi* may be due to the relatively low water temperatures usually experienced in Tasmania.

Rickettsial inclusions

The prevalence of rickettsial inclusions in *Crassostrea gigas* (1.4%) in Tasmania is similar to values reported for infection in *C. virginica* in the USA. In separate studies, values of 5% were reported by Meyers (1981) and by Gauthier et al. (1990) and 0.2-0.5% by Couch (1985).

Rickettsiales-like organisms have previously been reported from the digestive gland epithelium of *C. gigas* (Blackbourne et al., 1990; F. Kern, pers comm). Digestive gland inclusions were discovered in *C. gigas* in France during investigations of oyster mortalities, although it could not be shown that the mortalities were caused by the presence of the inclusions (Comps et al., 1977). Azevedo and Villalba (1991) discovered extracellular giant rickettsiae associated with the gill cilia of *C. gigas* in Spain.

There are no previously published reports of rickettsia in *Ostrea angasi.*

In Tasmania, rickettsial infections were geographically widespread in populations of both species of oyster.

The pattern of higher prevalence of rickettsial infections in winter observed in *C. gigas* during this study, are also reported by Meyers (1981) for infections in *C. virginica* and le Gall et al. (1991) note that mortalities of scallops (*Pecten maximus*), and prevalence of associated pathogenic rickettsia are highest during winter. This would indicate that environmental conditions are an important factor in these infections. In Tasmania, the higher prevalence of rickettsial inclusions in *C. gigas* on the east coast during winter could suggest a factor other than temperature (e.g. salinity) was involved as the east coast has consistently higher water temperatures than other areas of Tasmania. Alternatively, as higher numbers of rickettsial inclusions were seen in all areas during winter this may simply represent an annual trend.

Seasonal trends in prevalence of rickettsial inclusions in *O. angasi* were not apparent in this study. Similarly, seasonal trends were not observed in studies by Otto et al. (1979) and Couch (1985) of rickettsial infections of *C. virginica* in USA. These anomalies may be explained by the prevalence or behavior of as yet unknown vectors of these infections at different times of the year.

The discovery of rickettsial organisms in farmed shellfish in Tasmania and their widespread distribution is not surprising. These organisms are widespread in a variety of molluscan species, environments and geographic location. The absence of reports of these inclusions in Australian farmed shellfish is probably attributable to the lack of baseline research on the health of farmed oysters rather than their sudden appearance in shellfish populations.

In this study, four morphological types of rickettsial inclusions were identified in the digestive epithelium of Pacific oysters and two from the digestive tubule epithelium of flat oysters. Of these, 5 types (A_1, A_2, B from *C. gigas* and F and G from *O. angasi*) are morphologically similar to those commonly reported in the literature. Type C inclusions with a swirled appearance and positioned outside the digestive epithelium are rarely recorded in the literature. However, inclusions of a similar size and position to Type C inclusions in Tasmania was reported by Meyers (1981) in two clams (*Mercenaria mercenaria*) from USA.

Inclusions in the connective tissue, seen rarely in this study, were reported by Otto et al. (1979) while inclusions similar to those seen in the stomach epithelium in this study are previously unreported.

Although more than one morphological type of rickettsia was found in a population of oysters, only a single morphological type was detected in an individual oyster. Similar observations were made by Otto et al. (1979) and Meyers (1981). Different morphological types may represent different strains or species of rickettsial organisms. The detection of only one morphological type in an individual oyster may reflect the limitation of examining only one histological section per oyster.

Alternatively, different morphological types may represent pleomorphic forms of the same species/strain of rickettsial organisms with their appearance dependent on host species interactions or seasonal variation in host immune system or rickettsial life cycle. This tends to be supported by electron

microscope studies of two morphologically distinct inclusions of *O. angasi* which were ultrastructually similar. Both types of inclusion were seen in oysters collected from the same farm at different times of the year. Examination of the inclusions by electron microscopy revealed that the rickettsial organisms were identical in size and shape.

However, rickettsial inclusions in different species of oyster may be formed by different species of rickettsiales as was evidenced by electron microscope studies during this study in which rickettsial organisms in *C. gigas* and *O. angasi* were very different. While rickettsial organisms from *C. gigas* were very similar in shape to those reported in the literature (rod shaped, circular in cross section), rickettsial organisms in *O. angasi* were unusual in that they were star shaped in cross section. This shape was not caused by an artifact of the Davidson's fixation or deparrafinising as the star shape was also seen in organisms from *O. angasi* fixed in glutaraldehyde, and Pacific oyster inclusions fixed in Davidsons and deparaffinised were circular in cross section. The rickettsial inclusions in flat oysters also showed some similarities to chlamydial organisms previously described in other species of shellfish (Harshbarger et al., 1977). Dark patches noted in flat oyster rickettsia in this study were similar to features described as condensing bodies from chlamydia in hard clams (Harshbarger et al., 1977). However, the full range of developmental forms characteristic of chlamydia were not seen, and, in the absence of further corroborative evidence, the organisms have been classified as rickettsial organisms.

Non-membrane-bound vacuoles within rickettsial organisms from *C. gigas* in this study are also reported from inclusions in *Siliqua patula* in USA (Elston and Peacock, 1984) and *Tellina tenuis* in Scotland (Buchanan, 1978). Weiss and Moulder (1984) noted that this structure was often seen in mature rickettsia which have stopped growing. The function or cause of these vacuoles is not known although similar structures were noted by Anderson et al. (1965 op. cit. Buchanan, 1978) in a range of rickettsiae, mycoplasmas and chlamydiae.

"Blebs" or membrane outpocketings attached to the rickettsial organisms were noted by Elston and Peacock (1984) and Buchanan (1978). Buchanan (1978) stated that these were suggestive of replication of the rickettsial organisms by budding.

The observation of organisms undergoing binary fission is consistent with observations made by other researchers (Buchanan, 1978, Elston and Peacock, 1984). Reproduction by binary fission while keeping the membrane intact is characteristic of rickettsiales organisms (Weiss and Moulder, 1984).

Hyperparasitism of rickettsial organisms by phages causing destruction of infected rickettsial organisms has been reported by Harshbarger et al., (1977) and by Buchannan (1978). Particles are recorded by these authors as 50 nm and 66-68 nm respectively which is similar to the size of phages observed in *O. angasi* inclusions (50 nm). Despite the large number of reports of rickettsial organisms in molluscan hosts, reports by Harshbarger et al. (1977) and Buchanan (1978) are the only published accounts of hyperparasitism in rickettsial organisms infecting molluscan hosts. The occurrence of phages in rickettsial inclusions in flat oysters was associated with destruction of the prokaryote. Due to the amount of damage caused to the rickettsiales, the theory of Harshbarger et al. (1977) seems valid i.e. that phages act as a biological control in these infections.

The arrangement of the phages in paracrystalline arrays as described in Harshbarger et al. (1977) and Buchannan (1978) was not observed in the infections in Tasmania. Rather, phages were distributed throughout the cytoplasm of the rickettsial organism.

Rickettsial infections in *Crassostrea gigas* were often of light intensity whilst in *Ostrea angasi* infections were sometimes very heavy with most of the digestive tubule profiles in a histological section infected. Intensity of infections reported in the literature vary widely between shellfish species. The presence of these inclusions was not associated with disease or mortality in either species of oyster. However, in cases of heavy infections of flat oysters, the extra energetic burden of the parasites and the reduced ability of the host to absorb food may be detrimental. "Background" mortality of flat oysters may be due in part to these heavy infections especially in suboptimal conditions. Otto et al. (1979), Elston and Peacock (1984) and Goggin and Lester (1990) suggest that the effect of these infections may be increased under stressful conditions caused by culture methods or environmental factors.

The absence of a haemocytic response to these infections in both Pacific and flat oysters is consistent with observations of these inclusions in shellfish elsewhere

for both pathogenic and non pathogenic rickettsial infections (Gulka et al., 1983, Elston and Peacock, 1984, Buchanan, 1978).

In some cases, inclusions were seen in the lumen of infected *C. gigas* and *O. angasi* digestive tubules indicating expulsion of either the inclusion or the infected cell from the digestive epithelium. Elston and Peacock (1984) note that the host response of *Siliqua patula* to heavy rickettsiales-like infection comprises of rounding and detachment of infected epithelial cells and appears to be the only host response to the infections.

The ability of the oyster to slough and replace epithelial cells may be important in the survival of the host. It is reported in Weiss and Moulder (1984) that the reason why fleas are able to survive *Rickettsia typhi* infections better than louse, may be due to the ability of the flea to slough infected cells and renew its gut epithelial layer.

This action may also facilitate infection of new hosts as rickettsial inclusions are passed through the digestive tract and expelled with the faeces.

Many terrestrial and marine organisms carry or are infected by rickettsiales including insects, ticks, birds, terrestrial mammals, marine crustaceans (op. cit. Lauckner, 1983) and fish (Otto et al., 1979). Recently, a rickettsial disease of salmon was discovered in Chile (Fryer et al., 1990) and epitheliocystis (a disease caused by a prokaryotic organism) was reported for the first time in Australia (Langdon et al., 1991)

Until further work is done on the life cycles of these rickettsial organisms in shellfish and diseases of commercial and non-commercial marine organisms, the relationship between infections in molluscs and other marine or estuarine species can only be speculated.

There is a need for techniques to enable accurate identification of these organisms as some members of the Family Rickettsiales can cause serious illness and death in humans. As shellfish are often consumed raw, the potential public health consequences are obvious. The results of limited serological testing by Elston and Peacock (1984) suggests that the two groups i.e. rickettsia pathogenic for humans and rickettiales-like organisms in shellfish are serologically distinct and so eating infected shellfish will not cause rickettsial diseases in humans. In addition,

rickettsial inclusions in shellfish bear little resemblance (other than morphological features) to rickettsia infecting humans.

The major obstacle for studies of identification and infectivity trials of rickettsial infections is the inability to culture these organisms. Methods commonly employed in studies of mammalian rickettsial infections are not successful due to the lack of techniques for isolation and absence of molluscan or other suitable cell lines on which to grow these organisms (Elston and Peacock, 1984).

Buchannan (1978) was able to culture rickettsia from *Tellina tenuis* for a limited time in chick embryos and the salmonid rickettsia has been successfully cultured on fish cell lines (Lannan and Fryer, 1991). In the future the latter method may provide an alternative culture method for shellfish rickettsia in the absence of molluscan cell lines.

***Bonamia* sp**

During the period of this study, a parasite resembling *Bonamia* sp was discovered in farmed stocks of *O. angasi* in Tasmania. *Bonamia* sp has only recently been discovered in stocks of *O. angasi* in Australia. The first report was from Victoria in 1991 (Rawlins, pers. comm.) and in Tasmania, the parasite was diagnosed from wild stocks of *Ostrea angasi* in February, 1992 (Handlinger, pers comm).

Prevalence of *Bonamia* sp in cultured stocks of *O. angasi* in Tasmania (3-35%) was low when compared to stocks in Victoria (80%) (Rawlins, pers comm); New Zealand (up to 51%) (Hine, 1991a); and for *B. ostreae* infections of *O. edulis* in France (60%) (le Bec et al., 1991) but was similar to levels reported in Friedman et al. (1989) of 3-20% infection in *O. edulis* grown in California.

Further year round sampling is needed to determine the annual cycle of infection as samples were not taken regularly enough to accurately determine the time of greatest prevalence. In this study highest prevalence was seen in samples collected from Georges Bay in summer and autumn. Hence, the pattern of infection may be similar to *Bonamia* spp infections elsewhere where greatest prevalence and intensity occurs over the summer to autumn period (Rawlins, pers comm; Hine, 1991a; Poder et al., 1983).

During this survey, *Bonamia* sp was only found on a total of three leases from two bays (Georges Bay and Birchs Bay) in Tasmania. In all cases, farms infected with *Bonamia* sp had received stock from infected wild beds in Georges Bay.

The similar prevalence of *Bonamia* sp in oysters from lease A and B in Georges Bay probably reflects the prevalence of *Bonamia* sp in wild oysters used to stock the farms. On lease B, the significantly lower prevalence of *Bonamia* sp in naturally set oysters (10.0%) compared with adjacent wild harvested oysters (22.0%) may be due to the lower density of naturally set oysters, or the distance from the large source of infected oysters on the wild beds in Georges Bay some 2.5 - 3.5 km away.

The low prevalence of *Bonamia* sp in infected stock taken to Birchs Bay may indicate the slow rate of natural spread of the parasite and/or successful expulsion of parasites by the oysters. Also, these oysters were physically removed from the large stock of infected *O. angasi* in Georges Bay and placed in a location remote from large populations of wild or farmed *O. angasi*. Thus they were not continually exposed to large numbers of parasites in the water column.

Elevated levels of mortality, characteristically associated with the presence of *Bonamia* spp parasites, was not reported from farms with *Bonamia* sp infected oysters, nor from wild beds of *O. angasi* in Georges Bay (John Wilson, pers. comm.). Also, before commercial harvesting of oysters from Georges Bay was permitted, mortality and growth trials were conducted by the Division of Sea Fisheries (Hobart) on a sample of 300 oysters translocated from Georges Bay to Birchs Bay.

Information supplied by John Wilson (Division of Sea Fisheries, Hobart) showed that experimental stock held at a high intertidal height experienced 10% mortality and poor growth for a 6 month period from January - June. Stock held subtidally experienced 4% mortality and good growth over the same period.

Poor growth and increased mortality rates in intertidal stock may have been due to inappropriate culture methods as *O. angasi* is not tolerant of exposure to air as the time available for feeding is reduced (Anon, 1989). However, it is probable that a proportion of these translocated oysters were infected with *Bonamia* sp. The stress of being placed intertidally may have decreased the resistance of the oysters to their *Bonamia* sp infections. The role of stress in exacerbating

Bonamia spp infections has been well documented. Poder et al. (1982) found that mortalities of infected *O. edulis* were higher in intertidal areas and Hudson and Hill (1991) noted that resistance to *Bonamia* was decreased by stress due to exposure, handling, low temperatures and fluctuations in salinity.

The intensity of infection and the pathology associated with *Bonamia* sp in Tasmanian stocks of *O. angasi* is very different to that described in the literature for infection by *Bonamia* sp in New Zealand (Dinamani et al., 1987) or Victoria (Rawlins, pers. comm.), or *Bonamia ostreae* in Europe (Farley et al., 1988).

In this study, intensity of infection was low, with few *Bonamia* sp in the lesions. A maximum of 3 parasites were observed in a single haemocyte. Characteristically, *Bonamia* spp parasites are abundant in the tissue and up to 10 parasites have been recorded per haemocyte (Dinamani et al., 1987; Balouet et al., 1983).

The pathology typically associated with the presence of *Bonamia* spp has been described as a severe systemic infiltration of haemocytes in the connective tissue around the digestive tubules, gut and gills (Katkansky et al., 1969; Balouet et al., 1983; Friedman et al., 1989; Hine, 1991a). Parasites were correspondingly found throughout the tissue both intracellularly (within the haemocytes) and extracellularly (Farley et al., 1988). In addition, Dinamani et al. (1987) noted that parasites located beneath the basal membrane of the digestive tubules rarely migrated into or between the digestive epithelial cells.

The pathology associated with *Bonamia* sp in Tasmanian *O. angasi* of localised haemocytic response around the digestive gland or the gills, few extracellular parasites and location of parasites primarily within the digestive epithelium (either in the digestive cells or in haemocytes invading the digestive epithelium), is then, unusual. Systemic reactions typical of other *Bonamia* spp infection were rarely observed. Rawlins (pers. comm.) also reports the presence of focal haemocytic reaction in flat oysters from Victoria primarily during winter, although the typical pattern of systemic haemocytic reaction predominated during summer or autumn, the time of greatest mortality.

Therefore, the localised haemocytic reaction may be explained, in part, by a species specific reaction to the parasite.

Alternatively, oysters which exhibit a localised haemocytic response and "contain" parasites within epithelial borders may be less susceptible or more resistant to *Bonamia* sp infections.

Resistance to the acetosporan parasite *Haplosporidium nelsoni* (MSX) by *Crassostrea virginica* has been demonstrated by experimental studies involving selective breeding of oysters and exposure of successive generations to the pathogen (Haskin and Ford, 1979). Stocks of oysters selected for resistance have significantly higher survival rates that non selected stocks.

The pathology associated with the presence of the MSX parasites in selected stocks of *C. virginica* consists of localised haemocytic response with parasites usually confined within the epithelium (in this case the gill). Also, proliferation and the development of the parasites through their life stages are arrested (Ford, 1988). In comparison, the disease in unselected stocks is characterised by systemic haemocytic reaction, rapid proliferation of parasites and development of all life stages of the parasite.

A pattern of mortality and pathology similar to the selected resistant *C. virginica* stocks was observed in *Bonamia* sp infected *O. angasi* in Tasmania. Reported mortality was low in infected stocks and the host response consisted of localised haemocytic reaction primarily in the gut and gill epithelium where the parasites were most commonly found. In addition, numbers of parasites per infected cell were low and extracellular parasites were rarely seen, indicating a reduction in proliferation of the parasites.

Based on the comparisons between *Bonamia* sp infections in *O. angasi* in Tasmania with *Bonamia* spp infections in other species of shellfish, and, with the pattern of mortality, pathology, and proliferation of MSX in resistant stocks of *C. virginica*, the pattern of *Bonamia* sp infection in *O. angasi* in the Tasmanian populations studied is consistent with infection of a resistant or less susceptible stock of *O. angasi* to *Bonamia* sp.
It should be noted however, that these are preliminary observations made on oysters originating from a single population of *O. angasi* in Tasmania and the associated pathology may be different in oysters experiencing unfavourable or stressful conditions.

There is some evidence for resistance to *Bonamia ostreae* in *Ostrea edulis*. Preliminary results of experimental infections of *O. edulis* with *Bonamia ostreae* suggest that the F1 progeny of oysters with heavy infections are less susceptible to *Bonamia ostreae* (Hervio et al., 1992)

The pathology of *Bonamia* sp in *O. angasi* in Tasmania also tends to indicate that *Bonamia* sp has been present in Tasmania for a number of years and its recent discovery in Tasmania does not represent a newly acquired infection. Characteristically, newly acquired *Bonamia ostreae* infections are characterised by high mortality and systemic infection as has occurred in France, England and Ireland (Poder et al., 1982; Hudson and Hill, 1991; Mc Ardle et al., 1991).

The presence of *Bonamia* sp in Tasmania had been overlooked due to a combination of low numbers of samples examined, unusual pathology associated with the disease, low numbers of parasites in infected oysters, absence of gross sign associated with the disease and absence of reports of elevated mortality.

A technique for rapid diagnosis of *Bonamia* spp involving smears of heart tissue (Hine, pers. comm.) was not suitable for infections in Tasmania due to low numbers of parasites circulating in the haemolymph and diagnosis could only be made by histological examination. This, combined with absence of macroscopic signs of the disease in infected oysters, meant choosing an infected oyster/s and selecting a patch of tissue including *Bonamia* sp parasites for electron microscopy was very difficult and time consuming. For these reasons electron microscope studies undertaken during this study were unsuccessful.

Hine (1991b) and Rawlins (pers. comm.) have both observed light and dense forms of the parasite differentiated by light and electron microscopy. The relative proportions of each form changes throughout the year. During this study, only dense forms were seen in histological sections. This may have been due however, to the low numbers of oysters sampled and inconsistent sampling throughout the year. Further year round sampling of known infected populations may reveal a similar cycle of parasite development in Tasmania.

The spread of *Bonamia* sp (and many other shellfish diseases) throughout the world is associated with movements of stock rather than a natural spread of the parasite through dispersion (Farley, pers. comm.; Hudson and Hill, 1991; Elston et al., 1986). In Tasmania, the experience of translocation of (apparently

healthy) *Bonamia sp infected stock* between bays has stressed the importance of health testing before stocks are transferred between bays in relatively close proximity.

In Tasmania, the greatest risk of disease transfer in the future is by movements of infected *O. angasi* from infected to uninfected areas. Species of non-ostreid oysters eg *C. gigas* are not susceptible to the disease and it is thought that there is little risk in transfer of *Bonamia* sp with movements of *C. gigas* from *Bonamia* sp infected to uninfected areas. Transmission via parasites in the mantle fluid of Pacific oysters is thought to be unlikely due to large numbers of parasites needed for an infective dose. Hervio et al. (1992) found that between 100 000 and 1 000 000 *B. ostreae* parasites were needed to establish significant prevalence of infection in *O. edulis*. However, should infected flat oysters be attached to the shell of *C. gigas* being translocated, there would be a risk of transmission of *Bonamia* sp.

The source of the parasite in Tasmania, if introduced, is unknown although a number of potential sources exist. Flat oysters were relayed from New Zealand during the early 1900's (Dartnall, 1969). Also, fouling organisms carried on ships hulls may have included infected flat oysters. However, it is possible that Tasmania is included in the natural distribution of *Bonamia* sp and the parasite has been here as long as *Ostrea angasi*.

***Perkinsus* spp**

The negative results of testing for the pathogen *Perkinsus* sp supports the findings of earlier surveys for this disease in Tasmania (Goggin et al., 1988 and Goggin et al., 1989) which, similarly, yielded negative results. Relatively low water temperatures in Tasmania may be unfavourable for *Perkinsus* spp growth and development. Lester and Davis (1981) found only dead *Perkinsus olensi* in infected abalone held at 15°C and live parasites from similarly infected animals held at 20°C. High water temperatures are correlated with increases in infection intensity of *P. marinus* infecting *Crassostrea virginica* (Crosby and Roberts, 1990).

Ciliates

Ancistrocoma sp is one of the most common organisms associated with oyster tissues and have been reported from surveys of shellfish health world-wide (Blackbourne et al., 1990; Couch, 1985; Dinamani, 1986; Gauthier et al., 1990). Their presence in two species of oysters in Tasmania confirms their widespread distribution.

Levels of *Ancistrocoma* sp in the digestive gland of Tasmanian oysters is similar to that reported by Gauthier et al. (1990) in *C. virginica* (15.8%) and Dinamani (1986) in *C. gigas* in New Zealand (7-60%), but are higher than Newman (1971) (<1%) and Meyers (1981) (2.7%) for *C. virginica*.

In Tasmania, infections were light and occurred in healthy oysters. Consequently, *Ancistrocoma* sp is considered a commensal. The presence of these ciliates is not usually associated with disease or mortality (Meyers, 1981; Gauthier et al., 1990) although Couch (1985) notes heavy infection may block digestive tubules.

Ciliates are also common inhabitants of oyster gills and have been recorded in surveys by Newman (1971), Meyers (1981), Blackbourne et al. (1990).

In Tasmania, two types of gill ciliates and an unidentified organism were recorded from low numbers of *C. gigas*. *Trichodina* sp has previously been recorded from *C. gigas* in Canada. Organisms, similar to *Sphenophyra* sp and an unidentified organism in Tasmanian Pacific oysters were noted during surveys of shellfish health in USA (Newman, 1971; Couch, 1985) and New Zealand (Dinamani, 1986).

The observed intensity of infections was low, perhaps artificially, as these organisms are usually lost from the gills during fixation and histological processing. Limited host pathology was associated with these organisms. Xenomas sometimes associated with *Sphenophyra* sp (Couch, 1985) were not seen during this study.

The absence of any gill organisms in flat oysters may be due to the low numbers of flat oysters sampled, or the resistance of flat oysters to these infections.

These three organisms are thought to be of little consequence to oyster health in Tasmania as they were not associated with host pathology, disease or mortality.

Turbellarians

The prevalence of turbellarians in *C. gigas* (<1%) in Tasmania was very low when compared with figures of 70 and 88% reported by Jennings and Philips (1978) for infections of *Paravortex scrobicluariae* and *P. cardii* respectively, but are similar to values reported by Meyers (1981) of (1.4% & 2.9%) for infections in *C. virginica*. Turbellarians have also previously been recorded from *C. gigas* in Canada (Blackbourne et al., 1990)

Their low prevalence in *C. gigas* in Tasmania may be due to the intertidal habitat of Pacific oysters. Low numbers of trematodes in guts of *S. commercialis* from Australia are attributed to the intertidal environment of these oysters which limits the time available for infection by these organisms (Wolf et al., 1987).

The location of the turbellarians in the lumen of the intestine is similar to that reported by Jennings and Philips (1978) for *Paravortex scrobiculariae* and *P. cardii* and by Meyers (1981) for infection of *C. virginica* by an unidentified turbellarian.

Localised thinning of the host epithelial cells was noted at sites of attachment and in some cases host tissue was seen in the buccal cavity of the worms. It is unlikely that this localised damage to the intestinal epithelium is associated with a parasitic mode of life. Rather the worms are probably using the epithelium as a site of attachment. The majority of turbellarians associated with molluscs are considered commensals (Jennings, 1971).

These organisms are thought to be of little consequence to the health of Tasmanian oysters due to their low prevalence and absence of associated disease or mortality.

Copepods

Pseudomyicola spinosus

In *C. gigas* and *O. angasi* in Tasmania, prevalence of *Pseudomyicola spinosus* (3.2 and 3.7% respectively), is similar to that reported from mussels in the Hobart region (2.9%) by Pregenzer (1983). Up to 50% prevalence was reported from mussels on mainland Australia (Pregenzer, 1983) and 16-54% reported by Dinamani (1986) for infections of *C. gigas* in New Zealand.

Pregenzer (1983) noted that *Pseudomyicola spinosus* was more abundant in warmer waters, which would explain the relatively low prevalences in the cool waters of Tasmania, and why, during this survey, significantly higher numbers were seen on the east coast during summer.

In cases where the copepod had breached the digestive epithelium, it appeared from the degenerate internal structure of the copepod and the intense haemocytic response, that the oyster was able to kill the copepod.

Low intensity infections in Tasmanian oysters were not considered harmful to the host. Heavy infections with associated severe localised pathology may however, cause some metabolic stress to the host especially under unfavourable conditions. However, *P. spinosus* have not been associated with disease or elevated mortality in Tasmania and are considered commensal organisms.

Gill copepods

Specimens of copepods in the gills of *C. gigas* were too degenerate to identify. Gill copepods (*P. ostrea*) are reported from *C. gigas* in Canada (Blackbourne et al., 1990) and an unidentified copepod from *C. virginica* in Long Island (Meyers, 1981) similarly in low numbers.

The degenerate structure of these gill copepods indicated successful destruction by the oyster. Infected oysters appeared healthy and the low intensity and prevalence of these infection indicates they are not of significance to oyster health in Tasmania.

Polychaetes

Of the three spionid polychaete species found in shellblisters in *C. gigas* during this study, *Polydora websteri* had previously been associated with shellblister formation in *C. gigas* in Tasmania (Skeel, 1979). Skeel (1979) found *Polydora hoplura* and *Boccardia chilensis* only on the outside of the shell and it was suggested they were commensal species of little commercial significance. Their presence in shellblisters in this study may represent an opportunistic infection of an empty shellblister or a true shellblister forming association. If the latter is true, then these species should also be considered as parasitic although they are not as common as *P. websteri.*

It is probable that the non-spionid polychaete species recorded from shellblisters do represent opportunistic infections as only polychaetes of the family Spionidae have been recorded form shellblisters in the literature (Skeel 1979, Cole and Waugh, 1958). Members of these other families are common inhabitants of estuarine areas and probably moved into an empty blister during low tide or transport of oyster samples to the laboratory.

Shellblister infections caused by spionid polychaetes have been associated with extensive mortality in Sydney Rock oysters in New South Wales (Whitlegge, 1890; Roughley, 1925). However, even if infections are not severe enough to cause mortality, oysters may be unmarketable because of the unsightly appearance and the risk of breaking a blister during opening or cooking, or growth rates may be reduced (Cole and Waugh, 1957). By raising oysters off the benthos and holding stocks intertidally, the problem in New South Wales has been greatly reduced, although cultivation is restricted to intertidal areas (Skeel, 1979).

Exposure of oysters to the air during the tidal cycle desiccates the worm in its blister and it will either die or move out of the blister. In cases of high silt load in the water, oysters may be covered in a thick layer of mud at low tide, preventing desiccation of the worms. Pregenzer (1983) in a survey of symbionts of mussels in southern Australian waters found the highest infestation levels of spionid polychaetes where mussels were held in silty water or on the benthos.

Problems caused by shellblisters in Tasmania were rare and increased prevalence of blisters and 2% increase in mortality occurred in cases where racking holding

oysters off the bottom had sunk causing oysters to be submerged for a greater part of the tidal cycle (oyster grower, pers. comm.).

Mortality due to shellblisters was rarely experienced in Tasmania probably due to the off-bottom growing methods used and the fast growth rate of the Pacific oyster which is able to contain the worm by rapidly laying down shell. In comparison, Sydney rock oysters have a slower growth rate and may be less able to contain the worm by deposition of shell.

Lauckner (1983) suggests that the differences between the effect these infections have on various populations of shellfish may be due to host specific responses to infection.

Watery condition

Oysters of a poor or watery condition may be a sign of disease, poor nutrition or a postspawning state. Low levels of watery Pacific oysters were observed and the low mortality and absence of a detectable disease agent implicates factors other than disease as the cause. Because of the low numbers of oysters with this condition and absence of infectious agent this condition is not thought to pose an immediate risk to the health of Pacific oysters in Tasmania.

Changes in the histological appearance of Pacific oysters

In Pacific oysters it appears that the stage of gonadal development is strongly correlated with changes visible in histological sections.

High numbers of oysters with "full" connective tissue occurred at the time of early gonadal development. Increases in connective tissue edema occurred at the time of gonad maturation and spawning, and was apparent in post spawned oysters. This pattern was also seen by Meyers (1981) in *C. virginica* in USA where connective tissue edema occurred at, or soon after spawning.

Connective tissue is the site of glycogen (carbohydrate) storage in oysters and figures significantly in the reproductive cycle as carbohydrate is the major respiratory substrate during gametogenesis (Mann, 1978 op. cit. Perdue and Erickson, 1984) and is used as a precursor for the production of gametes

(Gabbott, 1975 op. cit. Perdue and Erickson, 1984). The pattern observed in this study indicates a build-up of glycogen during the early stages of gamete development and depletion of these energy reserves at the time of maturation and spawning followed by a recovery to normal levels some time after spawning.

Similarly, Perdue and Erickson (1984) found that in *C. gigas* in Washington, levels of carbohydrate decreased during gonad maturation and spawning and recovered after spawning. A study of glycogen levels in *C. gigas* in Tasmania by Kent (1990) also found that levels were low at or soon after spawning but recovered somewhat later, although the degree of recovery varied between sites with two sites showing persistent poor levels into winter many months after spawning.

In the southeast, high levels of connective tissue oedema continued until April, 1992 and reflects the poor condition of these oysters reported by growers at this time. Poor condition in the bay from which the April 1992 samples were taken, may be due to reduced food availability caused by overstocking of the bay with oysters, or reduced nutrient input. The main river running into this bay has been dammed and rainfall has been unseasonally low.

This pattern of connective tissue edema in relation to gonad development and spawning was more obvious in the second year of sampling in all areas except the east coast. It is not known why this occurred, but may be related to increased food availability or local environmental conditions during the first year of sampling. Local variations in environmental conditions and food availability may also explain the variations between areas at other times of the year.

The pattern of increased diffuse and focal haemocytic infiltration at the time of spawning or soon after was also observed by Meyers (1981) in *C. virginica* and Dinamani (1986) in *C. gigas*.

Haemocytes perform a number of functions in oysters including digestion and transport of food, resorption of unspent gonad material and defense against parasites and pathogens. Hence in spawning and post spawning oysters, it would be expected that haemocytic activity in all of these functions would be increased.

Increased haemocytic infiltration is considered to be a sign of poor condition in oysters (Meyers, 1981; Dinamani, 1986) and Couch (1985) notes systemic haemocytic infiltration is associated with stress caused by starvation, spawning or exposure to chemicals.

High levels of localised haemocytic infiltration on the east coast observed in postspawned oysters may also be caused by high levels of *Pseudomyicola spinosus* at this time. A localised haemocytic reaction was associated with the presence of this parasite in this study, and it was most abundant in summer, conincident with the time of spawning.

Digestive tubule atrophy has not clearly been linked with gametogenic cycles in oysters in the past. Meyers (1981) found that the pattern of digestive tubule atrophy in relation to spawning cycles was different in two populations of oysters studied. Couch (1985) noted seasonal trends in digestive tubule atrophy in *C. virginica* although these were not related to the time of spawning.

In this study, digestive tubule atrophy was correlated with stage of gonadal development, with increased atrophy seen in oysters with regressed gonads. As digestive tubule atrophy is often linked with poor condition or physiological stress the pattern seen in this study may reflect the generally poor condition of oysters (indicated by increased connective tissue oedema and haemocytic infiltration) at this time.

Digestive tubule atrophy is also thought to represent a metabolically inactive state of affected digestive tubules. The correlation of increased atrophy in lower salinity waters may be due to reduced feeding rate. Quayle (1988) noted that in Pacific oysters in Canada held at salinities lower than optimal levels (25-35%), feeding rate was decreased and oysters lost condition. Feeding rate is also reduced in conditions of high silt load (Quayle, 1988). An increase in turbidity during periods of rain and hence reduced salinity may also decrease feeding rate, in turn producing atrophied digestive tubules.

Studies by Gauthier et al. (1990) also found a correlation between digestive tubule atrophy in *C. virginica* and salinity although this was not attributed to metabolic stress at lower salinities. Rather it was attributed to pathology associated with an increase in the incidence of *Perkinsus* sp at lower salinities.

In this study, brown cell abundance was also correlated with salinity with higher levels of brown cells seen during conditions of lower salinity. Gauthier et al. (1990) did not observe this trend in *C. virginica*. Brown or pigment cells are are often associated with poor or diseased condition (Gauthier et al., 1990; Stein and Mackin, 1955 op. cit. Sparks, 1985) are indicative of abnormal fat metabolism possibly due to disease (Mackin, 1962 op. cit. Lauckner, 1983) and it is generally accepted that they are also involved in lysozyme digestion of foreign material and cellular debris.

An explanation for the increase in brown cell abundance in lower salinity water may reflect a metabolically altered state of the oysters, or that as digestive tubule epithelium is also reduced or shed at lower salinities, brown cells are more active in digestion of the waste cellular material.

The pattern of conincident occurrence of conditions indicative of good or poor health has been observed by many authors (Meyers, 1981; Couch, 1985; Dinamani, 1986; Gauthier et al., 1990).

There are many possible etiologies for these tissue conditions including stress related to gonad maturation and spawning, exposure to toxins, infection by a pathogen or local environmental conditions. Hence, these influences should be considered during studies of changes in histological appearance of oyster tissue.

From these studies, the pattern and probable causes of changes in the histological appearance of an apparently healthy population of *C. gigas* in Tasmania has been defined. Hence "abnormal" changes in response to a disease or exposure to a toxin or pollutant can be more easily differentiated.

Significance of Findings to the Tasmanian Oyster Industry

Perhaps the most significant finding of this study, is the absence of parasites or pathogens in farmed stocks of Pacific oysters. The organisms which were associated with the tissue of Pacific oysters were not associated with disease or mortality and are considered commensals.

The oyster industry in Tasmania is almost exclusively reliant upon the culture of the Pacific oyster and the presence of a disease or pathogen in Pacific oyster stocks causing mortality or poor condition would be devastating for the industry.

Establishment of a "disease free status" for Tasmanian Pacific oysters has enabled export of live Pacific oysters to USA for the restaurant trade, thus expanding markets for local growers.

Also information on the health status can be used for improved management of cultured stocks. Due to the benign nature of the commensal organisms and their widespread distribution throughout the state, it is not necessary to restrict movements of Pacific oysters between areas or bays. However, it should be noted that the present practises of distribution of nursery stock from one or two bays to farms throughout Tasmania, and the widespread transfers of stock between farms in different areas could quickly spread a disease throughout the state if not detected at an early stage. Indeed, these practises may account for the widespread distribution of commensal organisms seen in this study.

The second significant finding of this study was the important data collected concerning *Bonamia* sp in farmed stocks of *O. angasi* in Tasmania. *Bonamia* sp is well known as a pathogen of *Tiostrea chilensis* in New Zealand and flat oysters in Victoria, Australia. Although high mortality was not reported from infected stocks in Tasmania, further study is needed to determine the impact of this parasite for Tasmanian *O. angasi* populations.

In order to minimise the risk of transferring *Bonamia* sp to uninfected areas, management strategies of restricting movements of flat oysters are appropriate. Preliminary results indicate that *Bonamia* sp is relatively widespread in wild populations of flat oysters on the east and south east coasts of Tasmania, although some bays within these regions are apparently uninfected (Handlinger, pers. comm.). For export certification purposes, it may be impossible to claim a *Bonamia* sp free status for a single bay within a *Bonamia* sp infected region. However, for the management of stocks within Tasmania and to ensure sites of potential flat oyster cultivation, movements of flat oysters from infected to apparently uninfected bays should be restricted.

The impact of *Bonamia* sp on the Tasmanian oyster industry as a whole has been limited due to low numbers of flat oysters cultivated in Tasmania. A program to harvest wild flat oysters from Georges Bay to other parts of the state, however, has been severely disrupted.

Findings of this study also have significance for shellfish pathologists and managers. By defining the commensal fauna and associated host response, and normal changes in the tissue in "healthy" and a few "diseased" oysters, the cause of disease or mortality can be more easily assessed in the future.

Also, the disease risks associated with accidental or intentional introductions of marine organisms and their associated commensals and parasites can be more accurately assessed.

CHAPTER 5: SUMMARY CHAPTER

During the period October 1990 - April 1992, a study was undertaken to assess the health of farmed oyster stocks in Tasmania. The study was based primarily on Pacific oysters (*Crassostrea gigas*) as it is this species on which the Tasmanian oyster industry is based. A second species, the flat oyster (*Ostrea angasi*) was also studied although less intensively.

Oysters were collected on a near monthly basis from leases in each of 4 growing areas in Tasmania. Samples were examined histologically for the presence of pathogens, parasites and commensals and in Pacific oysters the histological appearance of the non reproductive tissues was assessed. A total of 5290 Pacific oysters and 630 flat oysters were examined during the study.

The results of the study indicate that Tasmanian stocks of farmed oysters have relatively few disease problems when compared with exploited stocks of oysters in other parts of Australia or the world.

Pacific oysters were free of any prescribed or potential pathogen. A number of commensal organisms were associated with the tissues including a viral infection of the gametes, rickettsial inclusions, two species of ciliate, two protozoans of unknown taxonomy, a turbellarian and two types of copepod. Shellblisters caused by spionid polychaetes were found in low numbers and were rarely associated with tissue damage or mortality.

Flat oysters were infected with similar commensal organisms including a viral inclusion, rickettsial inclusions, one species of ciliate and a crustacean. In addition, a pathogen *Bonamia* sp was found in some stocks. *Bonamia* sp has previously been associated with high mortalities and severe pathology in infected stocks in other parts of the world. In Tasmania, elevated mortality was not reported from farms with infected stock and the disease was characterised by low numbers of parasites and focal accumulations of haemocytes in the gut and gill. In Victoria and New Zealand where mortalities are experienced (Rawlins, pers. comm.; Hine 1991a) the disease is associated with large numbers of parasites and systemic infiltration of haemocytes. In all cases in Tasmania, infected farms had received *Bonamia* sp infected stock from wild beds in Georges Bay.

In addition to information on the parasites and commensals of the two oyster species, the condition of the non-reproductive tissues of Pacific oysters were assessed. Changes were related to the stage of gametogenesis and/or salinity. Conditions indicative of poor general health (connective tissue oedema, focal and diffuse haemocytic infiltration and digestive tubule atrophy) occurred at the time of or soon after spawning. These changes were reversible and in most cases the oysters recovered good histological appearance during autumn or winter. Storage of glycogen prior to spawning in spring (September/October) was evident. Increases in digestive tubule atrophy and brown cell abundance tended to occur in conditions of lower salinity.

This study has provided valuable baseline data regarding the seasonal and geographic distribution of commensal organisms in Tasmanian farmed oysters and normal changes in the histological appearance of Pacific oysters in apparently healthy populations.

In the event of a disease or increased mortality, such baseline information is useful to distinguish normal commensal organisms and pathology from pathogens, parasites or abnormal pathology. Hence the cause of a disease can be more quickly diagnosed and action taken to prevent its spread and/or limit its impact.

This study has also provided preliminary information on the occurrence of the pathogen *Bonamia* sp in farmed *O. angasi* in Tasmania, its distribution and associated pathology.

Through knowledge of local commensals, parasites and diseases, the risk and potential impact associated with the introduction of exotic species or non-endemic populations of oysters or other shellfish and their associated fauna can be more easily assessed.

It is important to maintain the disease-free status of Tasmanian Pacific oysters for certification of live seed oysters for relaying to other Australian states, to ensure access to international markets for live oysters, and to reduce the risk of losses of stocks in Tasmania due to disease.

Maintenance of a disease-free status can be achieved by:- strict control on the importation of potentially infective material e.g. live shellfish or ballast water;

maintenance of good management and growing techniques e.g. off bottom culture to reduce mudworm, low density stocking of bays to reduce stress and the removal of poor oysters during cultivation; and continued monitoring of farmed stocks including hatchery and nursery stocks.

The existing system of distribution of Pacific oyster seed and spat from a limited number of sources in Tasmania, and the widespread practices of movement of pre-market oysters between bays makes the industry potentially vulnerable to a disease. Although disease problems have not been encountered thus far, if a disease was introduced, it could be spread quickly throughout the state.

Thus it is important for the Tasmanian oyster industry that potential sources of disease are strictly controlled especially in the hatchery and nursery areas.

The risks of disease transfer with movements of stock has been highlighted by the movement of *Bonamia* sp to (perhaps) previously uninfected areas during the period of this study. Management strategies can be used to limit the impact of this disease on Tasmanian *O. angasi* populations. Although *Bonamia* sp is relatively widespread in wild populations of *O. angasi* in Tasmania and is considered endemic to the state (Handlinger, pers. comm.), it is possible that some areas/bays are free of *Bonamia* sp. Local restrictions on movements of *O. angasi* from infected to apparently uninfected areas/bays could restrict the distribution and impact of the disease on commercial stocks of *O. angasi* in Tasmania.

BIBLIOGRAPHY

Alderman, D.J. and Jones, G.B., 1971. Shell disease of oysters. Ministry of Agriculture, Fisheries and Food; Fishery investigations, Series II, vol 26, no 8, 20pp.

Anonymous, 1989. The biology and farming of oysters in South Australia. Sth Aust. Fisheries Research Branch, Adelaide, Sth Aust., Oct. 1989, 37 pp.

Azevedo, C. and Villalba, A., 1991. Extracellular giant rickettsiae associated with bacteria in the gill of *Crassostrea gigas* (Mollusca, Bivalvia). J. Invertebr. Path., 58: 75-81.

Balouet, G., Poder, M. and Cahour, A., 1983. Haemocytic parasitosis: morphology and pathology of lesions in the French flat oyster, *Ostrea edulis* L. Aquaculture, 34: 1-14.

Blackbourne, J., Nishimura, D.J.H., Bower, S.M., Meyer, G.R., 1990. Report on shellfish mortalities and certifications conducted since March 1990 and ninth update on survey of parasites and symbionts of Manila clams (*Tapes philippinarum*) and oysters (*Crassostrea gigas*) from various localities in British Columbia including a summary of oyster (*C. gigas*) diseases occurring on the coast of British Columbia. (unpublished reports) Pacific Biological Station, Nanaimo, 37 pp.

Blake, J.A., 1969. Reproduction and larval development of *Polydora* from northern New England (Polychaeta: Spionidae). Ophelia, 7: 1-63.

Blake, J.A. and Evans, J.W., 1973. *Polydora* and related genera as borers in mollusk shells and other calcerous substrates. The Veliger, 15: 235-249.

Bougrier, S., Tige, G., Bachere, E. and Grizel, H., 1986. *Ostrea angasi* accimatization to French coasts. Aquaculture, 58: 151-154.

Bower, S., 1987. Pathogenicity and host specificity of *Labyrinthuloides haliotidis* (Protozoa: Labyrinthomorpha), a parasite of juvenile abalone. Can. J. Zool., 65: 2008-2012.

Bower, S., 1988. Circumvention of mortalities caused by Denman Island oyster disease during mariculture of Pacific oysters. Am. Fish. Soc. Spec. Publ., 18: 246-248.

Brown, C., 1983. Bacterial diseases in bivalve larval cultures and their control. In: C. J. Berg (Editor), Culture of Marine Invertebrates. Hutchinson Ross Publishing Co., New York, 230-242.

Buchannan, J. S., 1978. Cytological studies on a new species of rickettsia found in association with a phage in the digestive gland of the marine bivalve mollusc, *Tellina tenuis* (da Costa). J. Fish Dis., 1: 27-43.

Bucke, D., Hepper, B., Key, D. and Bannister, R.C.A., 1984. A report on *Bonamia ostreae* in *Ostrea edulis* in the U.K.. I.C.E.S., Shellfish Committee. 7pp.

Burton, R.W., 1961. Distribution of oyster microparasites in Chesapeake Bay, Maryland, 1959-1960. Proc. Natl. Shellfish. Assoc., 52: 65-74.

Canning, E.U., 1986. Terminology, taxonomy and life cycles of Apicomplexa. Insect. Sci. Applic., 7: 319-325.

Cajaraville, M.P. and Angulo, E., 1991. Chlamydia-like organisms in digestive and duct cells of mussels from the Basque coast. J. Invertebr. Path., 58: 381-386.

Chagot, D.J., 1989. Caracterisation Morphologique et Fonctionnelle des Hemocytes D'*Ostrea edulis* et de *Crassostrea gigas*, Mollusques Bivalves. Etude *in vitro* de Leurs Interactions Avec le Protozoaire *Bonamia ostreae* (Acetospora). These, Laboratoire de Pathologie Comparee de l'Ecole Pratique des Hautes Etudes (Illeme Section), Montpellier, 72pp.

Cheng, T.C., 1988. Stategies employsed by parasites of marine bivalves to effect successful establishment in hosts. Am. Fish. Soc. Spec. Publ., 18: 112-129.

Cheng, T.C. and Combes, C., 1990. Influence of environmental factors on the invasion of molluscs by parasites: with special reference to Europe. In: F. di Castri, A.J. Hansen and M. Debussche (Editors), Biological Invasions in Europe and the Mediterranean Basin. Kluver Academic Publishers, Dordrecht, pp 307-332.

Cole, H.A. and Waugh, G.D., 1958. The problem of stunted growth in oysters. J. Cons. perm. int. Explor. Mer, 24: 355-365.

Comps, M., 1988. Epizootic diseases of oysters associated with viral infections. Am. Fish. Soc. Spec. Publ., 18: 23-37.

Comps, M., Bonami, J. and Vago, C., 1977. Pathologie des invertebres - Mise en evidence d'une infection rickettsienne chez les Huitres. C.R. Acad. Sci., Paris, 285, (Ser D): 427-429.

Couch, J.A., 1985. Prospective study of infectious and noninfectious diseases in oysters and fishes in three Gulf of Mexico estuaries. Dis. Aquat. Org., 1: 59-82.

Couch, J.A., 1988. Role of pathobiology in experimental marine biology and ecology. J. Exp. Mar. Biol. Ecol., 118: 1-6.

Crosby, M. and Roberts, C., 1990. Seasonal infection intensity cycle of the parasite *Perkinsus marinus* (and an absence of *Haplosporiduim* spp) in oysters from a South Carolina salt marsh. Dis. Aquat. Org., 9: 149-155.

Dartnall A. J., 1969. New Zealand sea stars in Tasmania. Pap. Proc. R. Soc. Tasm., 103: 53-55.

Dinamani, P., 1974. Reproductive cycle and gonadial changes in the New Zealand Rock oyster, *Crassostrea glomerata*. N.Z. J. Mar. Freshwater Res., 8: 39-65.

Dinamani, P., 1986. Potential disease-causing organisms associated with mantle cavity of Pacific oyster *Crassostrea gigas* in northern New Zealand. Dis. Aquat. Org., 2: 55-63.

Dinamani, P. and Hine, P.M. and Jones, J.B., 1987. Occurrence and characteristics of the haemocyte parasite *Bonamia* sp. in the New Zealand dredge oyster *Tiostrea lutaria*. Dis. Aquat. Org., 3: 37-44.

Dungan, C.F. and Elston, R.A., 1988. Histopathological and ultrastructural characteristics of bacterial destruction of the hinge ligaments of cultured juvenile Pacific oysters, *Crassostrea gigas*. Aquaculture, 72: 1-14.

Dungan, C.F., Elston, R.A. and Schiewe, M.H., 1989. Evidence for colonization and destruction of hinge ligaments in cultured juvenile Pacific oysters (*Crassostrea gigas*) by *Cytophaga*-like bacteria. Appl. Environ. Microbiol., 55: 1128-1135.

Elston, R.A., 1984. Prevention and management of infectious diseases in intensive mollusc husbandry. J. World. Maricult. Soc., 15: 284-300.

Elston, R.A., 1986. Occurrence of branchial rickettsiales-like infections in two bivalve molluscs, *Tapes japonica* and *Patinopecten yessoensis*, with comments on their significance. J. Fish. Dis., 9: 69-71.

Elston, R.A., 1990. Mollusc Diseases: Guide for the Shellfish Farmer. University of Washington Press, Washington, pp 73.

Elston, R.A., Beattie, J.H., Friedman, C., Hedrick, R. and Kent M.L., 1987. Pathology and significance of fatal inflammatory bacteraemia in the Pacific oyster, *Crassostrea gigas* Thunberg. J. Fish. Dis., 10: 121-132.

Elston, R.A., Farley, C.A. and Kent, M.L., 1986. Occurrence and significance of bonamiasis in European flat oysters *Ostrea edulis* in North America. Dis. Aquat. Org., 2: 49-54.

Elston, R.A., Kent, M.L. and Wilkinson, M.T., 1987. Resistance of *Ostrea edulis* to *Bonamia ostreae* infection. Aquaculture, 64: 237-242.

Elston, R.A. and Peacock M.G., 1984. A rickettsiales-like infection in the Pacific razor clam, *Siliqua patula*. J. Invertebr. Path., 44: 84-96.

Elston, R.A. and Wilkinson, M.T., 1985. Pathology, management and diagnosis of Oyster Velar Viral Disease (OVVD). Aquaculture, 48: 189-210

Farley, C.A., 1976. Ultrastructural observations on epizootic neoplasia and lytic virus infection in bivalve mollusks. Prog. Exp. Tumor Res., 20: 283-294.

Farley, C.A., 1978. Viruses and virus-like lesions in marine mollusks. Mar. Fish. Rev., 40: 18-20.

Farley, C.A., 1985. Viral gametocyte hypertrophy in oysters. ICES Identification Leaflet for Diseases and Parasites of Fish and Shellfish, no 25, Copenhagen, 5 pp.

Farley, C.A., 1988. Mass mortalities and infectious lethal diseases in bivalve mollusks and associations with geographic transfers of populations. J. Shellfish Res., 7: 554.

Farley, C.A., Banfield, W.G., Kasnic, G. (Jr), Foster, W.S., 1972. Oyster herpes-type virus. Science, 178: 759-760.

Farley, C.A., Wolf, P.H. and Elston, R.A., 1988. A long-term study of "microcell" disease in oysters with a description of a new genus, *Mikrocytos* (g. n.), and two new species, *Mikrocytos mackini* (sp. n.) and *Mikrocytos roughleyi* (sp. n.). Fish Bull., 86: 581-593.

Figueras, A.J. and Montes, J., 1988. Aber disease of edible oysters caused by *Martelia refringens*. Am. Fish. Soc. Spec. Publ., 18: 38-46.

Ford, S.E., 1988. Host parasite interactions in eastern oysters selected for resistance to *Haplosporidium nelsoni* (MSX) disease: survival mechanisms against a natural pathogen. Am. Fish. Soc. Spec. Publ., 18: 206-224.

Ford, S.E. and Haskin, H.H., 1982. History and epizooitiology of *Haplosporidium nelsoni* (MSX), an oyster pathogen in Delaware Bay, 1957-1980. J. Invertebr. Pathol., 40: 118-141.

Freeman, R.F.H., 1957. *Paravortex scrobiculariae* (Graff) in Great Britain. Nature, 180: 1213-1214.

Friedman, C.S. and Hedrick R.P., 1991. Pacific oyster nocardiosis: isolation of the bacterium and induction of laboratory infections. J. Invertebr. Pathol., 57: 109-120.

Friedman, C.S., McDowell, T., Groff, J., Hollibaugh, J., Manzer, D. and Hedrick, R., 1989. Presence of *Bonamia ostreae* among populations of the European flat oyster, *Ostrea edulis* Linne, in California, USA. J. Shellfish Res., 8: 133-137.

Fries, C. R. and Grant, D.M., 1991. Rickettsiae in gill epithelial cells of the hard clam *Mercenaria mercenaria*. J. Invertebr. Path., 57: 166-171.

Fries, C.R., Grau, S.B., and Tripp, M.R., 1991. Rickettsiae in the cytoplasm of gill epithelial cells of the soft shelled clam, *Mya arenaria*. J. Invertebr. Path., 57: 443-445.

Fryer, J.L., Lannan, C.N., Garces, L.H., Larenas, J.J., and Smith, P.A., 1990. Isolation of a rickettsiales-like organism from diseased Coho salmon (*Oncorhynchus kisutch*) in Chile. Fish Pathol., 25: 107-114.

Garland, C.D., Nash, G.V., Sumner, C.E. and McMeekin, T.A., 1983. Bacterial pathogens of oyster larvae (*Crassostrea gigas*) in a Tasmanian hatchery. Austn. J. Mar. Freshw. Res., 34: 483-487.

Gauthier, J.D., Soniat, T.M. and Rogers, J.S., 1990. A parasitological survey of oysters along salinity gradients in coastal Louisiana. J. World Aqua. Soc., 21: 105-115.

Goggin, C.L. and Lester, R.J.G., 1988. Survey of Tasmanian molluscs for *Perkinsus* sp. 20 June - 1 July, 1988. Report to Dover Fisheries, Dover, Tasmania (unpublished manuscript), pp 5.

Goggin, C.L., Clark, A. and Lester R.J.G. 1989. Survey of Tasmanian molluscs for *Perkinsus* sp, 14-22 February, 1989. Report to Dover Fisheries, Royal Park, South Australia (unpublished manuscript), pp 5.

Goggin, C.L. and Lester, R.J.G., 1987. Occurrence of *Perkinsus* species (Protozoa, Apicomplexa) in bivalves from the Great Barrier Reef. Dis. Aquat. Org., 3: 113-117.

Goggin, C.L. and Lester R.J.G., 1990. Rickettsiales-like infection in the gills of *Tridacna crocea* from the Great Barrier Reef. J. Invertebr. Path., 56: 135-138.

Goggin, C.L., Sewell, K.B. and Lester, R.J.G., 1989. Cross-infection experiments with Australian *Perkinsus* species. Dis. Aquat. Org., 7: 55-59.

Grischkowsky, R.S. and Liston J., 1974. Bacterial pathogenicity in laboratory induced mortality of the Pacific oyster (*Crassostrea gigas*, Thunberg). Proc. Natl. Shellfish. Assoc., 64: 82-90.

Grizel, H., Miahle, E., Chagot, D., Boulo, V. and Bachere, E., 1988. Bonamiasis: A model study of diseases in marine molluscs. Am. Fish. Soc. Spec. Publ., 18: 1-4.

Gulka G., Chang, P.W. and Marti, K.A., 1983. Prokaryotic infection associated with a mass mortality of the sea scallop, *Placopecten magellanicus*. J. Fish Dis., 6: 355-364.

Harshbarger, J.C., Chang, S.C. and Otto, S.V., 1977. Chlamydiae (with phages), mycoplasmas, and rickettsiae in Chesapeake Bay Bivalves. Science, 196: 666-668.

Hartman, O., 1941. Some contributions to the biology and life history of Spionidae from California. Allan Hancock Pacific Expeditions 1932 - 40, 7: 289-323.

Haskin, H.H. and Ford, S.E., 1979. Development of resistance to *Minchinia nelsoni* (MSX) mortality in laboratory-reared and native oyster stocks in Delaware Bay. Mar. Fish. Rev., 41: 54-63.

Hayat, M.A., 1970. Principles and Techniques of Electron Microscopy; Biological Applications, Vol 1. van Nostrand Reinhold Company, New York.

Hervio, D., Bachere, E., Cochennec N., Boulo, V., Vuillemen, V., le Coguic, Y., Mazurine, J. and Mialhe, E., 1992. Establishment of an experimental infection protocol for the flat oyster *Ostrea edulis* with the intrahaemocytic protozoan parasite *Bonamia ostreae*: application in the selection of parasite resistant oysters. In: Hervio, D., 1992. Contribution a L'etude de *Bonamia ostreae* (Acetospora), protozoaire parasite de l'huitre *Ostrea edulis* (Bivalvia), et a l'analyse des interactions hote-parasite. These, Universite Blaise Pascal - Clarmont Ferrand, 170p.

Hine, P.M., 1991a. The annual pattern of infection by *Bonamia* sp. in New Zealand flat oysters, *Tiostrea chilensis*. Aquaculture, 93: 241-251.

Hine, P.M., 1991b. Ultrastructural observations on the annual infection pattern of *Bonamia* sp. in flat oysters *Tiostrea chilensis*. Dis. Aquat. Org., 11: 163-171.

Hine P.M., Wesney, B. and Hay, B.E., 1992. Herpesvirus associated with mortalities among hatchery-reared larval Pacific oysters *Crassostrea gigas*. Dis. Aquat. Org., 12: 135-142.

Hopkins, S.H., 1958. The planktonic larvae of *Polydora websteri* Hartman (annelida, Polychaeta) and their settling on oysters. Bull. Mar. Sci. Gulf. Caribb., 8: 268-277.

Howard, D.W. and Smith, C.S., 1983. Histological Techniques for Marine Bivalve Mollusks. NOAA Technical Memorandum NMFS-F/NEC - 25, Massacheusetts, 97pp.

Hudson, E.B. and Hill, B.J., 1991. Impact and spread of Bonamiasis in the UK. Aquaculture, 93: 279-285.

Jennings, J.B., 1971. Parasitism and commensalism in the Turbellaria. Adv. Parasitol., 9: 1-32.

Jennings, J.B. and Phillips, J.I., 1978. Feeding and digestion in three entosymbiotic graffillid rhabdocoels from bivalve and gastropod molluscs. Biol. Bull., 155: 542-562.

Katkansky, S.C., Dahlstrom, W.A. and Warner, R.W., 1969. Observations on survival and growth of the European flat oyster, *Ostrea edulis*, in California. Calif. Fish Game, 55: 69-74.

Kent, A., 1990. Investigation of changes in condition during spawning and recovery in the Pacific oyster, *Crassostrea gigas* (Thunberg, 1793) on three Tasmanian oyster farms. Masters Thesis, University of Tasmania, 24 pp.

Khan, M.A., 1956. Fine structure of *Ancistrocoma pelseneeri* (Chatton et Lwoff), a rhynchodine thigmotrichid ciliate. Acta. Protozoologica, 7: 29-47.

Kozloff, E.N., 1946. Studies of the Family Ancistrocomidae Chatton and Lwoff (Order Holotricha, Suborder Thigmotricha) III. *Ancistrocoma pelseneeri* Chatton

and Lwoff, *Ancistrocoma dissimilis* sp. nov., and *Hypocomagalma pholadidis* sp. nov. Biol Bull., 91: 189-199.

Langdon, J.S., Elliot, K. and Mackay, B., 1991. Epitheliocystis in the leafy sea-dragon. Aust. Vet. J., 68: 244.

Lannan, C.N. and Fryer, J.L., 1991. Recommended methods for inspection of fish for the salmonid rickettsia. Bull. Eur. Ass. Fish Pathol., 11: 135-136.

Lauckner, G., 1983. Diseases of Mollusca: Bivalvia. In O. Kinne (Editor), Diseases of Marine Animals, vol II: Bivalvia to Scaphopoda. Biologische Anstalt Helgoland, Hamburg, pp 477-879.

le Bec, C., Mazurie, J., Cochennec, N. and le Coguic, Y., 1991. Influence of *Crassotrea gigas* mixed with *Ostrea edulis* on the incidence of *Bonamia* disease. Aquaculture, 93: 263-271.

le Gall, G., Chagot, D., Mialhe, E. and Grizel, H., 1988. Branchial rickettsiales-like infection associated with a mass mortality of sea scallop *Pecten maximus*. Dis. Aquat. Org., 4: 229-232.

le Gall, G., Mialhe, E., Chagot, D. and Grizel, H., 1991. Epizootiological study of rickettsiosis of the Saint-Jacques scallop *Pecten maximus*. Dis. Aquat. Org., 16: 139-145.

Leibovitz, L., 1989. Chlamydiosis: a newly reported serious disease of larval and postmetamorphic bay scallops, *Argopecten irradians* (Lamarck). J. Fish Dis., 12: 125-136.

Leibovitz, L., Elston, R., Lipovosky, V.P. and Donaldson, J., 1978. A serious disease of larval Pacific oysters (*Crassostrea gigas*). Proc. 9th Annu. Meet. World Maricult. Soc., Atlanta, pp 603-615.

Lester, R.J.G., 1986. Field and laboratory observations on the oyster parasite *Martelia sydneyi*. In: M. Cremin, C. Dobson, and D. Moorhouse (Editors) Parasite Lives, University of Queensland Press, St Lucia, pp 33-40.

Lester, R.J.G. and Davis, G.H.G., 1981. A new *Perkinsus* species (Apicomplexa, Perkinsea) from the abalone *Haliotis ruber*. J. Invertebr. Path., 37: 181-187.

Lester, R.J.G., Goggin, C.L. and Sewell, K.B., 1990. *Perkinsus* in Australia. In: F.O. Perkins and T.C. Cheng (editors) Pathology in Marine Science. Acedemic Press, San Diego, pp 189-199.

Lipovsky, V.P. and Chew, K.K., 1972. Mortality of Pacific oysters (*Crassostrea gigas*): the influence of temperature and enriched seawater on oyster survival. Proc. Natl. Shellfish. Assoc., 62: 72-82.

Lom, J. and Kozloff, E.N., 1968. Observations on the ultrastructure of the suctorial tube of the Ancistrocomid ciliates. Folia. Parasitol., 15: 291-308.

Loosanoff, V.L. and Davis, H.C., 1963. Rearing of bivalve molluscs. In: F.S. Russel (Editor), Advances in Marine Biology, Vol 1. Academic Press, New York, pp 1-136.

Loosanoff, V.L. and Engle, J.B., 1943. *Polydora* in oysters suspended in the water. Biol. Bull., 85: 69-78.

Lunz, G.R., 1940. The annelid worm, *Polydora*, as an oyster pest. Science, 92: 310.

McArdle, J.F., McKiernan, F., Foley, H. and Jones, D.H., 1991. The current status of *Bonamia* disease in Ireland. Aquaculture, 93: 273-278.

Meyers, T.R., 1979. A reo-like virus isolated from juvenile American oysters (*Crassostrea virginica*). J. Gen. Virol., 43: 203-212.

Meyers, T.R., 1981. Endemic diseases of cultured shellfish of Long Island, New York: adult and juvenile American oysters (*Crassostrea virginica*) and hard clams (*Mercenaria mercenaria*). Aquaculture, 22: 305-330.

Miahle, E., Boulo, V., Elston, R., Hine, M., Montes, J., van Banning, P. and Grizel, H., 1988. Serological analysis of *Bonamia* in *Ostrea edulis* and *Tiostrea lutaria* using polyclonal and monoclonal antibodies. Aquat. Living Resour., 1: 67-69.

Newman, M.W., 1971. A parasite and disease survey of Connecticut oysters. Proc. Natl. Shellfish. Assoc., 61: 59-63.

Nicolas, J.L., Comps, M. and Cochennec, N., 1992. Herpes-like virus infecting Pacific oyster larvae, *Crassostrea gigas*. Bull. Eur. Ass. Fish. Pathol. 12: 11.

Otto, S.V., Harshbarger, J.C. and Chang, S.C., 1979. Status of selected unicellular eucaryote pathogens, and prevalence and histopathology in inclusions containing obligate procaryote parasites, in commercial bivalve mollusks from Maryland estuaries. Haliotis, 8: 285-295.

Page, L.A. and Cutlip, R.C., 1982. Morphological and immunological confirmation of the presence of chlamydiae in the gut tissues of the Chesapeake Bay clam, *Mercenaria mercenaria*. Curr. Microbiol., 7: 297-300.

Parry, G.D., Langdon, J.S. and Huisman, J.M., 1989. Toxic effects of a bloom of the diatom *Rhizosolenia chunii* on shellfish in Port Philip Bay, southeastern Australia. Mar. Biol., 102: 25-41.

Pass, D.A., Dybdahl, R. and Mannion, M.M., 1987. Investigations into the causes of mortality of the pearl oyster *Pinctada maxima* (Jamson), in Western Australia. Aquaculture, 65: 149-169.

Pass, D.A., Perkins F.P. and Dybdahl, R., 1988. Viruslike particles in the digestive gland of the pearl oyster (*Pinctada maxima*). J. Invert. Pathol., 51: 166-167.

Pauley, G.B., Chew, K.K. and Sparks, A.K., 1967. Experimental infection of oysters *Crassostrea gigas* with Thigmotrichid ciliates. J. Invertebr. Path., 9: 230-234.

Perdue, J.A. and Erickson, G., 1984. A comparison of the gametogenic cycle between the Pacific oyster *Crassostrea gigas* and the Suminoe oyster *Crassostrea rivularis* in Washington State. Aquaculture, 37: 231-237.

Perkins, F.O., 1988. Structure of protistan parasites found in bivalve molluscs. Am. Fish. Soc. Spec. Publ., 18: 93-111.

Perkins, F.O. and Wolf, P.H., 1976. Fine structure of *Martelia sydneyi* sp. n. - haplosporidan pathogen of Australian oysters. J. Parasitol., 62: 528-538.

Pichot, Y., Comps, M., Tige, G., Grizel, H. and Rabouin, M.A., 1979: Recherches sur *Bonamia ostreae* gen. n.. sp. n., parasite nouveau de l'huitre plate *Ostrea edulis* L.. Rev. Trav. Inst. Peches Marit., 43: 131-140.

Poder, M., Cahour, A. and Balouet, G., 1982. Hemocytic parasitosis in European oyster *Ostrea edulis* L.: Pathology and contamination. In: C.C. Payne and H.D. Burges (Editors), Invertebrate Pathology and Microbial Control. Proceedings of the International Coloquium on Invertebrate Pathology. XVth Annu. Meet. Soc. Invert. Pathol., September 6-10, 1982, University of Sussex, Brighton, pp 254-257.

Pregenzer, C., 1983. Survey of metazoan symbionts of *Mytilus edulis* (Mollusca: Pelecypoda) in Southern Australia. Aust. J. Mar. Freshw. Res., 34: 387-396.

Quayle, D.B., 1988. Pacific oyster culture in British Columbia. Can. Bull. Fish. Aquat. Sci., 218: 1-214.

Quick, J.A., 1972. Fluid thioglycollate medium assay of labyrinthomyxa parasites in oysters. Florida Dept Of Natl Resources Leaflet Series: Vol 6, Part 4, No 3, St Petersburg, 12pp.

Raghukumar, C. and Lande, V., 1988. Shell disease of rock oyster *Crassostrea cucullata*. Dis. Aquat. Org., 4: 77-81.

Ray, S.M., 1952. A culture technique for the diagnosis of infections with *Dermocystidium marinum* Mackin, Owen and Collier in oysters. Science, 166: 360-361.

Ray, S.M., 1966. A review of the culture method for detecting *Dermocystidium marinum*, with suggested modifications and precautions. Proc. Natl. Shellfish. Assoc., 54: 55-69.

Rogan, E., Culloty, S.C., Cross, T.F. and Mulcahy, M.F., 1991. The detection of *Bonamia ostreae* (Pichot et al., 1980) in frozen oysters (*Ostrea edulis* L.) and the effect of the parasite on condition. Aquaculture, 97: 311-315.

Roughley, T.C., 1925. The perils of an oyster. Austn. Mus. Magaz., 2: 277-284.

Sinderman, C.J., 1976. Oyster mortalities and their control. In FAO Technical Conference on Aquaculture (Kyoto, Japan, 1976), FAO-FIR: AQ/Conf/76/R. 34: 1-25.

Skeel, M.E., 1979. Shell-boring worms (Spionidae: Polychaeta) infecting cultivated bivalve molluscs in Australia. Proc. World Maricult. Soc., 10: 529-533.

Sparks, A.K., 1985. Synopsis of invertebrate pathology (exclusive of insects). Elsevier, Amsterdam, 423 pp.

Sprague, V., 1979. Classification of the Haplosporidia. Mar. Fish. Rev., 41: 40-44.

Stephen, D., 1978. Mud blister formation by *Polydora ciliata* in the Indian backwater oyster *Crassostrea madrasensis* (Preston). Aquaculture, 13: 347-350.

van Banning, P., 1985. Control of *Bonamia* in Dutch oyster culture. In: A.E. Ellis (Editor), Fish and Shellfish Pathology. Academic Press, London, pp 393-396.

van Banning, P., 1986. Case History of the *Bonamia ostreae* control in the Dutch oyster culture. In: C.P. Vivares, J. -R. Bonami and E. Jaspers (editors), Pathology in Marine Aquaculture. Europ. Aquacult. Soc. Spec. Publ. no 9, bredene, Belgium, pp, 23-27.

van Banning, P., 1990. The life cycle of the oyster pathogen *Bonamia ostreaee* with a presumptive phase in the ovarian tissue of the European flat oyster, *Ostrea edulis.* Aquaculture, 84: 189-192.

van Banning, P., 1991. Observations on bonamiasis in the stock of the European flat oyster, *Ostrea edulis*, in the Netherlands, with special reference to the recent developments in Lake Grevelingen. Aquaculture, 93: 205-211.

Wardle, W.J., 1980. Occurrence of the symbiotic rhabdocoele flatworm *Paravortex gemellipara* in Chesapeake Bay and Gulf of Mexico molluscs, with notes on its biology and geographic range. Estuaries, 3: 84-88.

Weiss, E. and Moulder, J.W., 1984. The Rickettsias and Chlamydias. In: N.R. Krieg and J.G. Holt (editors) Bergey's Manual of Systematic Bacteriology, Vol 1. Williams and Wilkins, Baltimore, pp 687-739.

Whitlegge, T., 1890. Report on the worm disease affecting the oysters on the coast of New South Wales. Austn. Mus. Records 1: 41-54.

Wolf, P.H., 1972. Occurrence of a haplosporidian in Sydney rock oysters (*Crassostrea commercialis*) from Moreton Bay, Queensland, Australia. J. Invertebr. Pathol., 19: 116-117.

Wolf, P.H., 1979. Life cycle and ecology of *Martelia sydneyi* in the Australian oyster, *Crassostrea commercialis*. Mar. Fish. Rev., 41: 70-72.

Wolf, P.H., Winstead, J.T. and Couch, J.A., 1987. *Proctoeces* sp (Trematoda: Digenea) in Australian oysters, *Saccostrea commercialis* and *Crassostrea amasa*. Trans. Am. Microsc. Soc., 106: 379-380.

APPENDIX 1

List of growning areas, farm locations and lease/permit numbers licenced for bivalve shellfish cultivation in Tasmania

AREA	LOCALITY	LEASE/PERMIT NO
AREA 1 Northwest and King Island	Big Bay (1) Big Bay (2) Big Bay (3) Duck Bay Port Sorell King Island	25,20,54 21,32 82 22, 23 95 38
AREA 2 East Coast	Moulting Bay Little Swanport Great Swanport (1) Great Swanport (2)	9,27,65 18,52,86 49,51 50
AREA 3 Southeast	Eaglehawk Neck/ Norfolk Bay Blackman Bay/ Dunalley Pipeclay (1) Pipeclay (2) Pittwater (1) Pittwater (2)	17,14,84 11,96,10,113 127 13,15,98,108 12,91,97 46,47,48 1,80,81
AREA 4 D'Entrecasteaux Channel and Bruny Island	Birchs Bay Hastings Bay Port Esperance Port Cygnet Long Bay Shoal Recherche Bay Great Bay Cloudy Bay Little Taylors Bay	45,57 5,6 36,66 136 3,41 35 39,42,69,70 4 59

APPENDIX 2

List of samples of *Crassostrea gigas* and *Ostrea angasi* collected from oyster farms in Tasmania during July 1990 - April 1992.

DATE	AREA	LOCALITY	LEASE/ PERMITNO	SAMPLE
13/7/90	SE	Pittwater	48	50 x 0+ *C. gigas*
				50 x 1+ *C. gigas*
19/7/90	NW	Big Bay	25	50 x 0+ *C. gigas*
24/7/90	CH	Birchs Bay	45	15 x 0+ *C. gigas*
				15 x 1+ *C. gigas*
1/8/90	EC	Little Swanport	86	50 x 1+ *C. gigas*
1/8/90	SE	Pittwater	47	50 x 0+ *C. gigas*
				50 x 1+ *C. gigas*
9/8/90	NW	Duck Bay	22	50 x O+ *C. gigas*
15/8/90	CH	Port Esperance	66	50 x 1+ *C. gigas*
27/8/90	EC	Moulting Bay	65	50 x O+ *C. gigas*
				50 x 1+ *C. gigas*
4/9/90	CH	Birchs Bay	45	25 x 4+ *O.angasi*
11/9/90	NW	Big Bay	21	50 x 1+ *C.gigas*
13/9/90	SE	Eaglehawk Neck	17	72 x 1+ *C.gigas*
				25 x 2+ *O.angasi*
14/9/90	CH	Long Bay Shoal	3	50 x 1+ *C.gigas*
				50 x 3+ *C.gigas*
3/10/90	EC	Great Swanport	49	50 x 0+ *C.gigas*
				50 x 1+ *C.gigas*
				25 x 2+ *O.angasi*
11/10/90	NW	Big Bay	82	50 x 1+ *C.gigas*
18/10/90	EC	Great Swanport	50	50 x 0+ *C.gigas*
				50 x 1+ *C.gigas*
17/10/90	CH	Little Taylors Bay	59	50 x 0+ *C .gigas*
				50 x 1+ *C.gigas*
18/10/90	SE	Pipeclay	12	50 x 0+ *C.gigas*
				50 x 1+ *C.gigas*
4/12/90	EC	Little Swanport	86	25 x 0+ *C.gigas*
			86	25 x 1+ *C.gigas*
			18	25 x 0+ *C.gigas*
			18	25 x 1+ *C.gigas*
7/12/90	SE	Pittwater	1	11 x 0+ *C.gigas*
			1	10 x 1+ *C.gigas*
			1	8 x 1+ *O.angasi*
			1	10 x 2+ *O.angasi*
			80	24 x 0+ *C.gigas*
			80	15 x 1+ *C.gigas*
			81	15 x 1+ *C.gigas*
10/12/90	CH	Long Bay Shoal	3/41	25 X 0+ *C.gigas*
			3/41	25 X 1+ *C.gigas*
12/12/90	NW	Big Bay	54	25 X 0+ *C.gigas*
			54	25 X 1+ *C.gigas*
			20	25 x 0+ *C.gigas*
			20	25 x 1+ *C.gigas*

DATE	AREA	LOCALITY	LEASE	SAMPLE
15/1/91	NW	Duck Bay	22	25 x 0+ *C.gigas*
			23	25 x 0+ *C.gigas*
14/1/91	SE	Pipeclay	13	12 x 0+ *C.gigas*
			13	13 x 1+ *C.gigas*
			15	13 x 0+ *C.gigas*
			15	12 x 1+ *C.gigas*
			108	13 x 0+ *C.gigas*
			108	12 x 1+ *C.gigas*
			98	12 x 0+ *C.gigas*
			98	13 x 1+ *C.gigas*
20/1/91	CH	Great Bay	39	13 x 0+ *C.gigas*
			39	16 x 1+ *C.gigas*
			42	12 x 0+ *C.gigas*
			42	12 x 1+ *C.gigas*
			70	15 x 0+ *C.gigas*
			70	15 x 1+ *C.gigas*
29/1/91	EC	Moulting Bay	65	20 x 0+ *C.gigas*
			65	20 x 1+ *C.gigas*
			27	10 x 1+ *O.angasi*
			27	10 x 3+ *O.angasi*
			9	15 x 0+ *C.gigas*
			9	12 x 1+ *C.gigas*
			9	10 x 2+ *O.angasi*
12/2/91	NW	Big Bay	21	25 x 0+ *C.gigas*
			32	25 x 0+ *C.gigas*
12/2/91	SE	Blackman Bay	10	10 x 0+ *C.gigas*
			11	11 x 0+ *C.gigas*
			11	15 x 1+ *C.gigas*
			11	10 x 1+ *O.angasi*
			11	15 x 3+ *O.angasi*
			96	10 x 0+ *C.gigas*
			96	15 x 1+ *C.gigas*
			113	10 x 0+ *C.gigas*
			113	15 x 1+ *C.gigas*
13/2/91	CH	Hastings Bay	6	12 x 1+ *O.angasi*
			6	20 x 0+ *C.gigas*
			6	15 x 1+ *C.gigas*
7/3/91	CH	Hastings Bay	5	20 x 0+ *C.gigas*
			5	25 x 1+ *C.gigas*
			5	5 x 4+ *C.gigas*
27/2/91	EC	Great Swanport	49	40 x 0+ *C.gigas*
5/3/91			49	40 x 1+ *C.gigas*
			49	10 x 1+ *O.angasi*
			49	10 x 2+ *O.angasi*

DATE	AREA	LOCALITY	LEASE	SAMPLE
8/4/91	CH	Recherche Bay	35	15 x 0+ *C.gigas*
			35	10 x 1+ *C.gigas*
			136	10 x 0+ *C.gigas*
			136	10 x 1+ *C.gigas*
		Little Taylors	59	15 x 0+ *C.gigas*
		Bay	59	10 x 1+ *C.gigas*
		Cloudy Bay	4	10 x 1+ *C.gigas*
11/4/91	SE	Pittwater	46	20 x 0+ *C.gigas*
			46	20 x 1+ *C.gigas*
			47	20 x 0+ *C.gigas*
			47	20 x 1+ *C.gigas*
			48	20 x 0+ *C.gigas*
			48	20 x 1+ *C.gigas*
16/4/92	EC	Great Swanport	50	50 x 0+ *C.gigas*
			50	50 x 1+ *C.gigas*
6/5/92	NW	Big Bay	82	50 x 1+ *C.gigas*
13/5/92	EC	Little Swanport	52	5 x 0+ *C.gigas*
			52	5 x 1+ *C.gigas*
			86	20 x 0+ *C.gigas*
			86	20 x 1+ *C.gigas*
			18	25 x 0+ *C.gigas*
			18	25 x 1+ *C.gigas*
14/5/92	CH	Birchs Bay	45	15 x 0+ *C.gigas*
			45	15 x 1+ *C.gigas*
			45	5 x 1+ *O.angasi*
			45	5 x 3+ *O.angasi*
14/5/91	CH	Birchs Bay	57	20 x 0+ *C.gigas*
			57	20 x 1+ *C.gigas*
21/5/91	SE	Eaglehawk	14	25 x 0+ *C.gigas*
		Neck/Norfolk	14	15 x 1+ *C.gigas*
		Bay	17	15 x 1+ *C.gigas*
			17	10 x 3+ *O.angasi*
			84	25 x 0+ *C.gigas*
			84	15 x 1+ *C.gigas*
22/5/91	NW	Big Bay	54	20 x 0+ *C.gigas*
			54	20 x 1+ *C.gigas*
			25	12 x 0+ *C.gigas*
			25	15 x 1+ *C.gigas*
			20	19 x 0+ *C.gigas*
			20	25 x 1+ *C.gigas*

DATE	AREA	LOCALITY	LEASE	SAMPLE
5/6/91	NW	Duck Bay	23	25 x 0+ *C.gigas*
			22	10 x 1+ *C.gigas*
			22	18 x 0+ *C.gigas*
12/6/91	SE	Pipeclay	12	20 x 0+ *C.gigas*
			12	17 x 1+ *C.gigas*
			91	15 x 0+ *C.gigas*
			91	17 x 1+ *C.gigas*
			97	15 x 0+ *C.gigas*
			97	17 x 1+ *C.gigas*
17/6/91	CH	Port Esperance	36	45 x 0+ *C.gigas*
			36	25 x 1+ *C.gigas*
			66	25 x 1+ *C.gigas*
2/7/91	EC	Moulting Bay	9	12 x 0+ *C.gigas*
			9	14 x 1+ *C.gigas*
			9	10 x 1+ *O.angasi*
			65	21 x 0+ *C.gigas*
			65	17 x 1+ *C.gigas*
17/7/91	EC	Moulting Bay	27	10 x 0+ *O.angasi*
			27	15 x 1+ *O.angasi*
3/7/91	NW	Big Bay	21	28 x 0+ *C.gigas*
			32	27 x 0+ *C.gigas*
9/7/91	EC	Great Swanport	49	40 x 0+ *C.gigas*
			49	40 x 1+ *C.gigas*
			49	10 x 1+ *O.angasi*
			49	10 x 2+ *O.angasi*
11/7/91	SE	Pittwater	1	20 x 0+ *C.gigas*
			1	15 x 1+ *C.gigas*
			1	20 x 1+ *O.angasi*
			1	15 x 2+ *O.angasi*
9/7/91			80	20 x 0+ *C.gigas*
	SE	Pittwater	80	15 x 1+ *C.gigas*
			81	15 x 1+ *C.gigas*
9/7/91	CH	Long Bay Shoal	3	20 x 0+ *C.gigas*
			3	20 x 1+ *C.gigas*
		Simpsons Bay	41	20 x 0+ *C.gigas*
			41	20 x 1+ *C.gigas*
17/9/91	EC	Great Swanport	50	50 x 0+ *C.gigas*
			50	50 x 1+ *C.gigas*
18/9/91	SE	Pipeclay	13	15 x 0+ *C.gigas*
			13	16 x 1+ *C.gigas*
			15	16 x 0+ *C.gigas*
			15	15 x 1+ *C.gigas*
			98	17 x 0+ *C.gigas*
			98	12 x 1+ *C.gigas*
			108	14 x 0+ *C.gigas*
			108	14 x 1+ *C.gigas*
28/9/91	NW	Big Bay	82	50 x 1+ *C.gigas*
7/10/91	CH	Little Taylors	59	50 x 0+ *C.gigas*
		Bay	59	25 x 1+ *C.gigas*
		Cloudy Lagoon	4	25 x 1+ *C.gigas*

DATE	AREA	LOCALITY	LEASE	SAMPLE
9/10/91	NW	Big Bay	20	17 x 0+ *C.gigas*
			20	17 x 1+ *C.gigas*
			25	17 x 0+ *C.gigas*
			25	17 x 1+ *C.gigas*
			54	17 x 0+ *C.gigas*
			54	17 x 1+ *C.gigas*
22/10/91	SE	Blackman Bay	10	10 x 0+ *C.gigas*
			11	10 x 0+ *C.gigas*
			11	15 x 1+ *C.gigas*
			11	10 x 1+ *O.angasi*
			11	10 x 2+ *O.angasi*
			96	10 x 0+ *C.gigas*
			96	15 x 1+ *C.gigas*
			113	10 x 1+ *C.gigas*
			113	15 x 1+ *C.gigas*
28/10/91	EC	Little Swanport	18	20 x 0+ *C.gigas*
			18	20 x 1+ *C.gigas*
			52	10 x 0+ *C.gigas*
			52	10 x 1+ *C.gigas*
			86	20 x 0+ *C.gigas*
			86	20 x 1+ *C.gigas*
29/10/91	CH	Great Bay	39	17 x 0+ *C.gigas*
			39	17 x 1+ *C.gigas*
			42	17 x 0+ *C.gigas*
			70	17 x 0+ *C.gigas*
			70	17 x 1+ *C.gigas*
			42	7 x 1+ *C.gigas*
16/11/91	NW	Duck Bay	22	31 x 0+ *C.gigas*
			22	12 x 1+ *O.angasi*
			22	12 x 2+ *O.angasi*
			23	25 x 0+ *C.gigas*
19/11/91	SE	Pittwater	46	20 x 0+ *C.gigas*
			46	20 x 1+ *C.gigas*
			47	20 x 0+ *C.gigas*
			47	20 x 1+ *C.gigas*
			48	20 x 0+ *C.gigas*
			48	20 x 1+ *C.gigas*
20/11/91	CH	Hastings Bay	5	25 x 0+ *C.gigas*
			5	20 x 1+ *C.gigas*
			6	25 x 0+ *C.gigas*
			6	20 x 1+ *C.gigas*
27/11/91	EC	Georges Bay	9	20 x 0+ *C.gigas*
			9	15 x 1+ *C.gigas*
			9	13 x 2+ *O.angasi*
			27	20 x 1+ *O.angasi*
			27	20 x 2+ *O.angasi*
			65	20 x 0+ *C.gigas*
			65	15 x 1+ *C.gigas*

DATE	AREA	LOCALITY	LEASE	SAMPLE
20/1/92	SE	Eaglehawk/	14	10 x 1+ *C.gigas*
		Norfolk Bay	17	10 x 1+ *C.gigas*
			17	10 x 3+ *O.angasi*
			84	20 x 0+ *C.gigas*
			84	10 x 1+ *C.gigas*
		Blackman Bay	127	20 x 0+ *C.gigas*
			127	10 x 1+ *C.gigas*
21/1/92	CH	Recherche Bay	35	50 x 0+ *C.gigas*
			35	50 x 1+ *C.gigas*
30/1/92	KI	King Island	38	30 x 1+ *C.gigas*
5/2/92	EC	Great Swanport	50	80 x 0+ *C.gigas*
			50	60 x 1+ *C.gigas*
6/2/92	NW	Port Sorell	95	25 x 0+ *C.gigas*
			95	25 x 1+ *C.gigas*
		Big Bay	21	25 x 0+ *C.gigas*
			32	25 x 1+ *C.gigas*
14/2/92	SE	Pipeclay	12	25 x 0+ *C.gigas*
			12	17 x 1+ *C.gigas*
			97	25 x 0+ *C.gigas*
			97	17 x 1+ *C.gigas*
			91	17 x 1+ *C.gigas*
20/2/92	EC	Great Swanport	49	40 x 0+ *C.gigas*
			49	20 x 1+ *C.gigas*
			49	20 x 1+ *O.angasi*
			49*	10 x 2+ *O.angasi*
			49	20 x 2+ *O.angasi*
			51	20 x 1+ *C.gigas*
19/2/92	CH	Birchs Bay	45	16 x 0+ *C.gigas*
			45	15 x 1+ *C.gigas*
			45	11 x 1+ *O.angasi*
			45	15 x 3+ O.angasi
			57	25 x 1+ *C.gigas*
			57	25 x 0+ *C.gigas*
15/3/92	NW	Big Bay	82	50 x 1+ *C.gigas*

DATE	AREA	LOCALITY	LEASE	SAMPLE
16/3/92	NW	Big Bay	20	17 x 0+ *C.gigas*
			20	17 x 1+ *C.gigas*
			54	35 x 0+ *C.gigas*
			54	17 x 1+ *C.gigas*
			25	17 x 1+ *C.gigas*
19/3/92	SE	Pittwater	1	25 x 0+ *C.gigas*
			1	20 x 1+ *C.gigas*
			80	20 x 0+ *C.gigas*
			80	20 x 1+ *C.gigas*
			81	20 x 0+ *C.gigas*
			81	20 x 1+ *C.gigas*
27/3/92	EC	Little Swanport	18	20 x 0+ *C.gigas*
			18	20 x 1+ *C.gigas*
			52	10 x 0+ *C.gigas*
			52	10 x 1+ *C.gigas*
			86	20 x 0+ *C.gigas*
			86	20 x 1+ *C.gigas*
31/3/92	CH	Port Esperance	36	50 x 0+ *C.gigas*
			36	25 x 1+ *C.gigas*
			66	34 x 1+ *C.gigas*
10/4/92	NW	Duck Bay	22	25 x 0+ *C.gigas*
			22	25 x 1+ *C.gigas*
			23	25 x 0+ *C.gigas*
			23	25 x 1+ *C.gigas*
14/4/92	EC	Moulting Bay	9	25 x 0+ *C.gigas*
			9	25 x 1+ *C.gigas*
			9	60 x 2+ *O.angasi*
			27	60 x 1+ *O.angasi*
			27	60 x 2+ *O.angasi*
			65	25 x 0+ *C.gigas*
			65	25 x 1+ *C.gigas*
24/4/92	SE	Pipeclay	13	15 x 0+ *C.gigas*
			13	15 x 1+ *C.gigas*
			15	15 x 0+ *C.gigas*
			15	15 x 1+ *C.gigas*
			98	15 x 0+ *C.gigas*
			98	15 x 1+ *C.gigas*
			108	15 x 0+ *C.gigas*
			108	15 x 1+ *C.gigas*
7/5/92	CH	Long Bay Shoal	3	50 x 0+ *C.gigas*
			3	25 x 1+ *C.gigas*
			41	25 x 1+ *C.gigas*